THE UNIVERSITY of ADELAIDE

ACAD Australian Centre for Ancient DNA

BIOINFORMATICS AND SYSTEMS MODELLING

Ancient DNA

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Lecture overview

- Introduction to ancient DNA
- Sources of ancient DNA and post-mortem DNA decay
- Properties of ancient DNA
- Ancient DNA analysis

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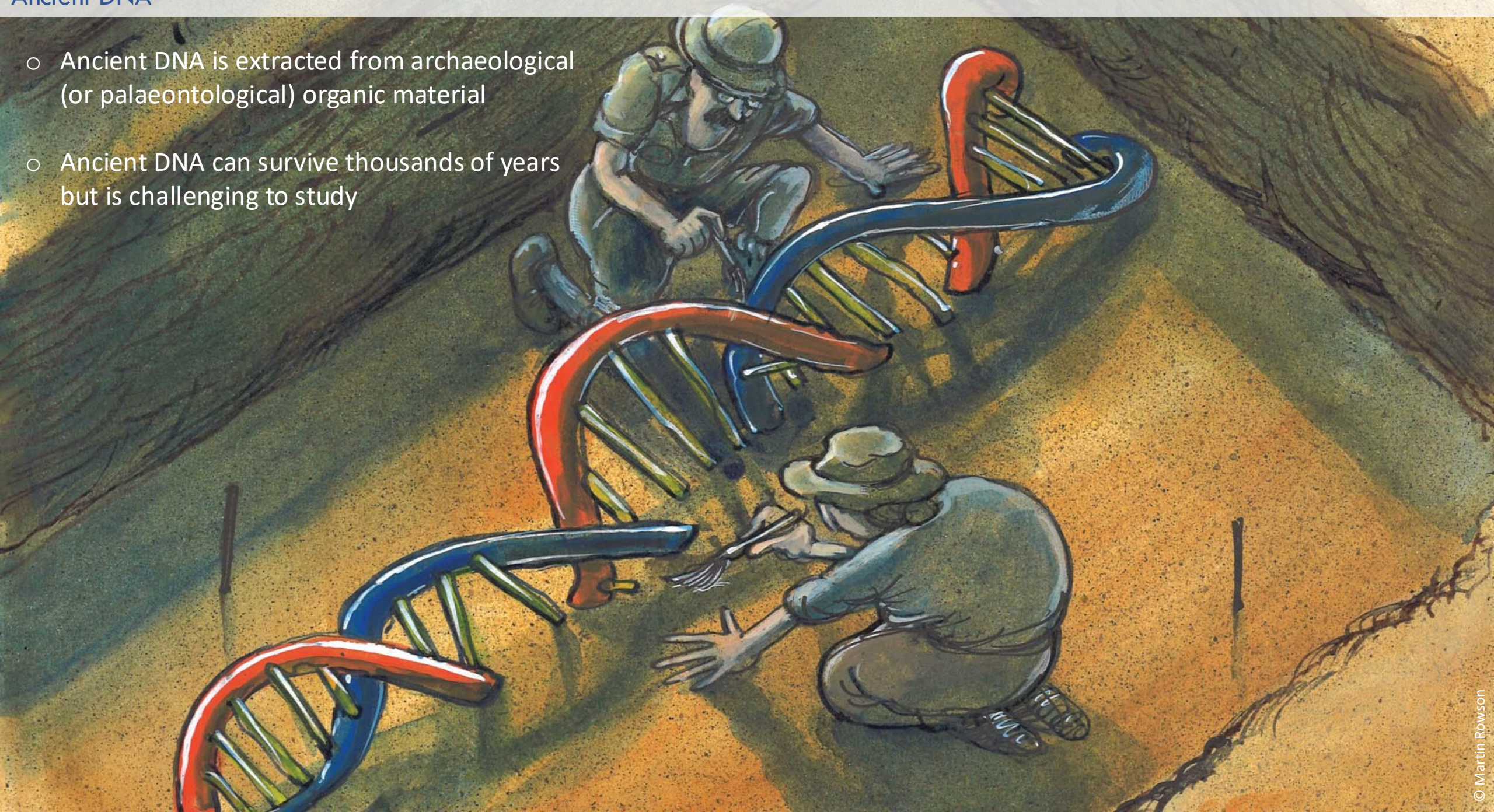
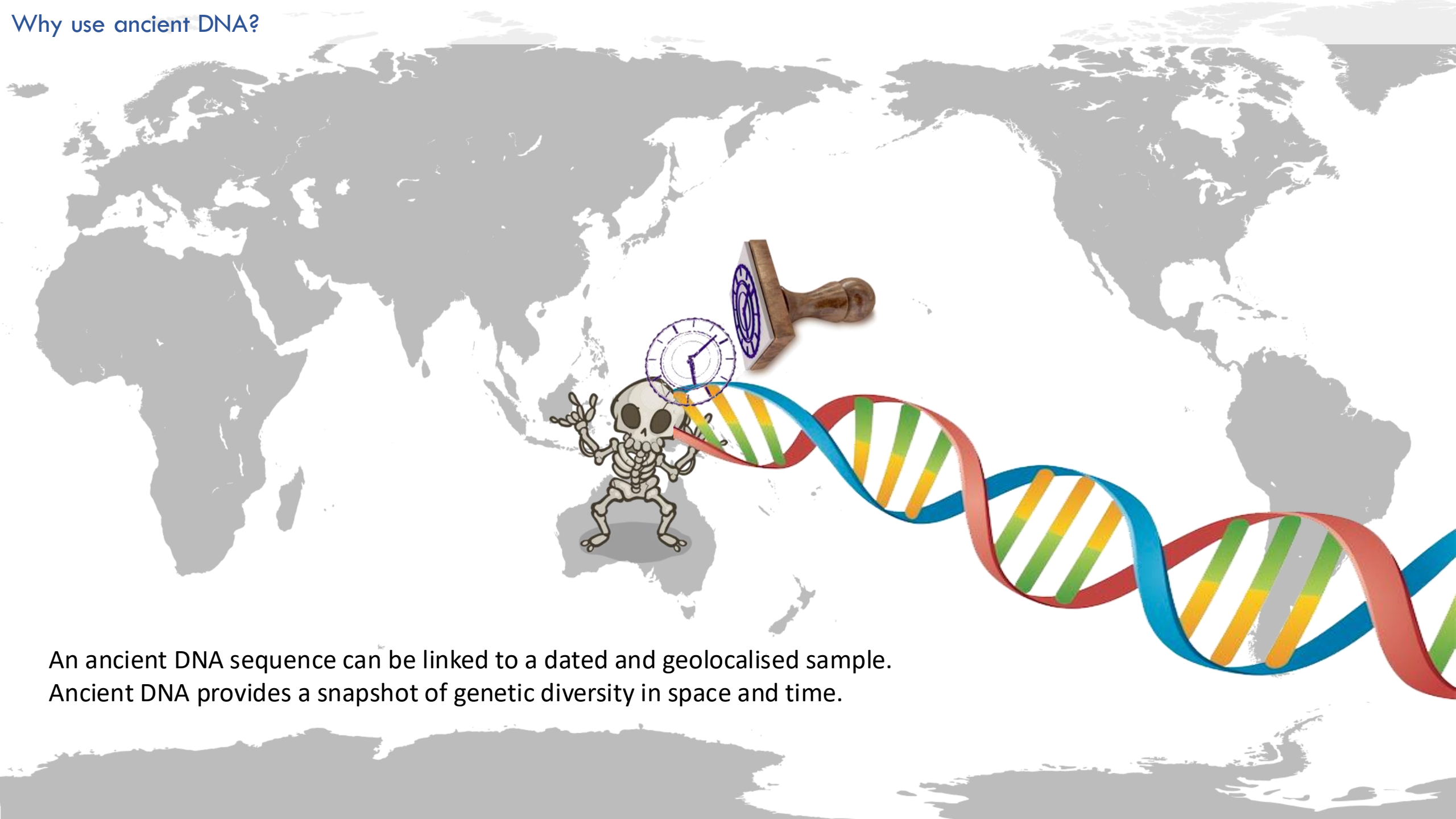
Ancient DNA

- Ancient DNA is extracted from archaeological (or palaeontological) organic material
- Ancient DNA can survive thousands of years but is challenging to study

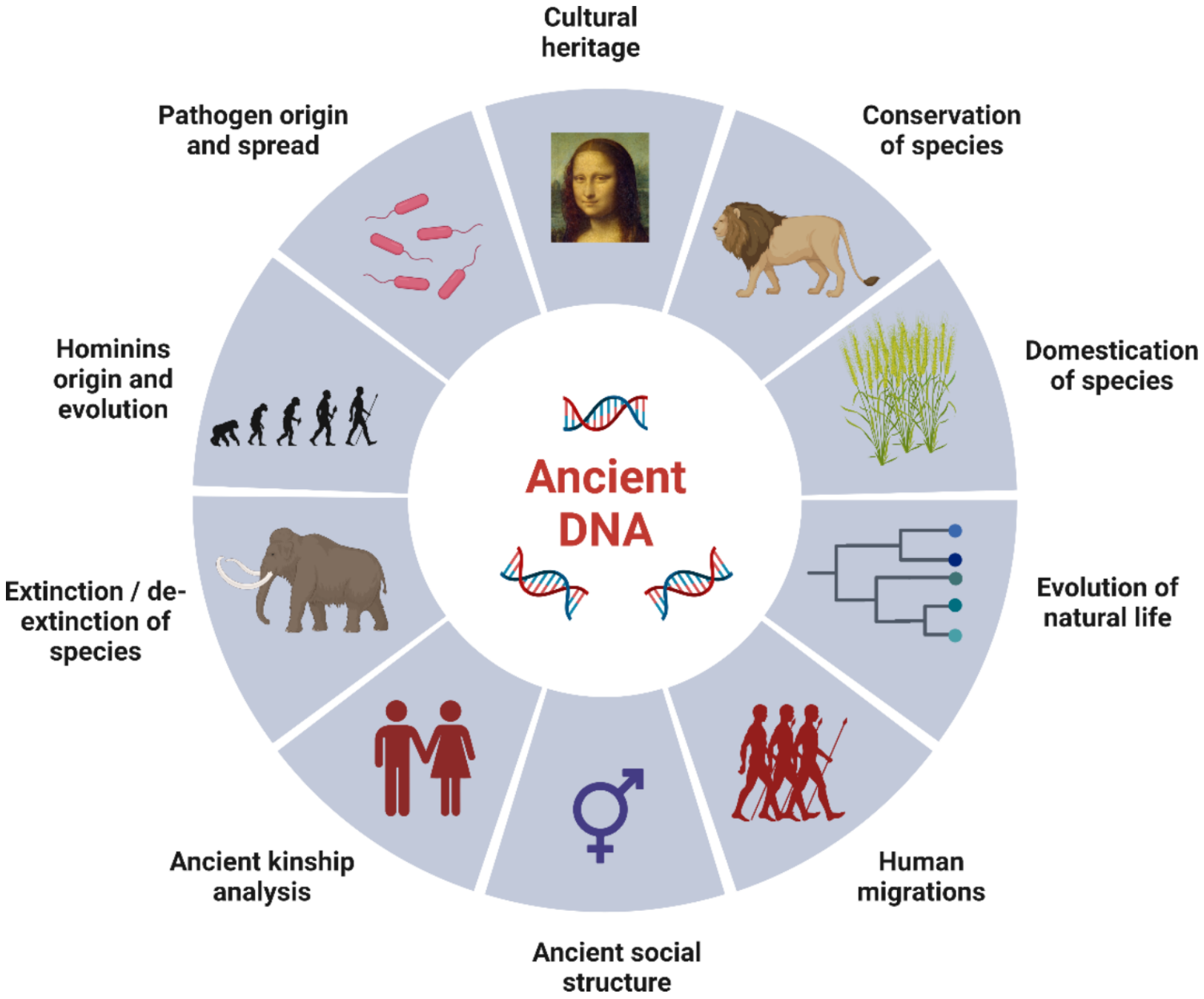


Why use ancient DNA?



An ancient DNA sequence can be linked to a dated and geolocalised sample.
Ancient DNA provides a snapshot of genetic diversity in space and time.

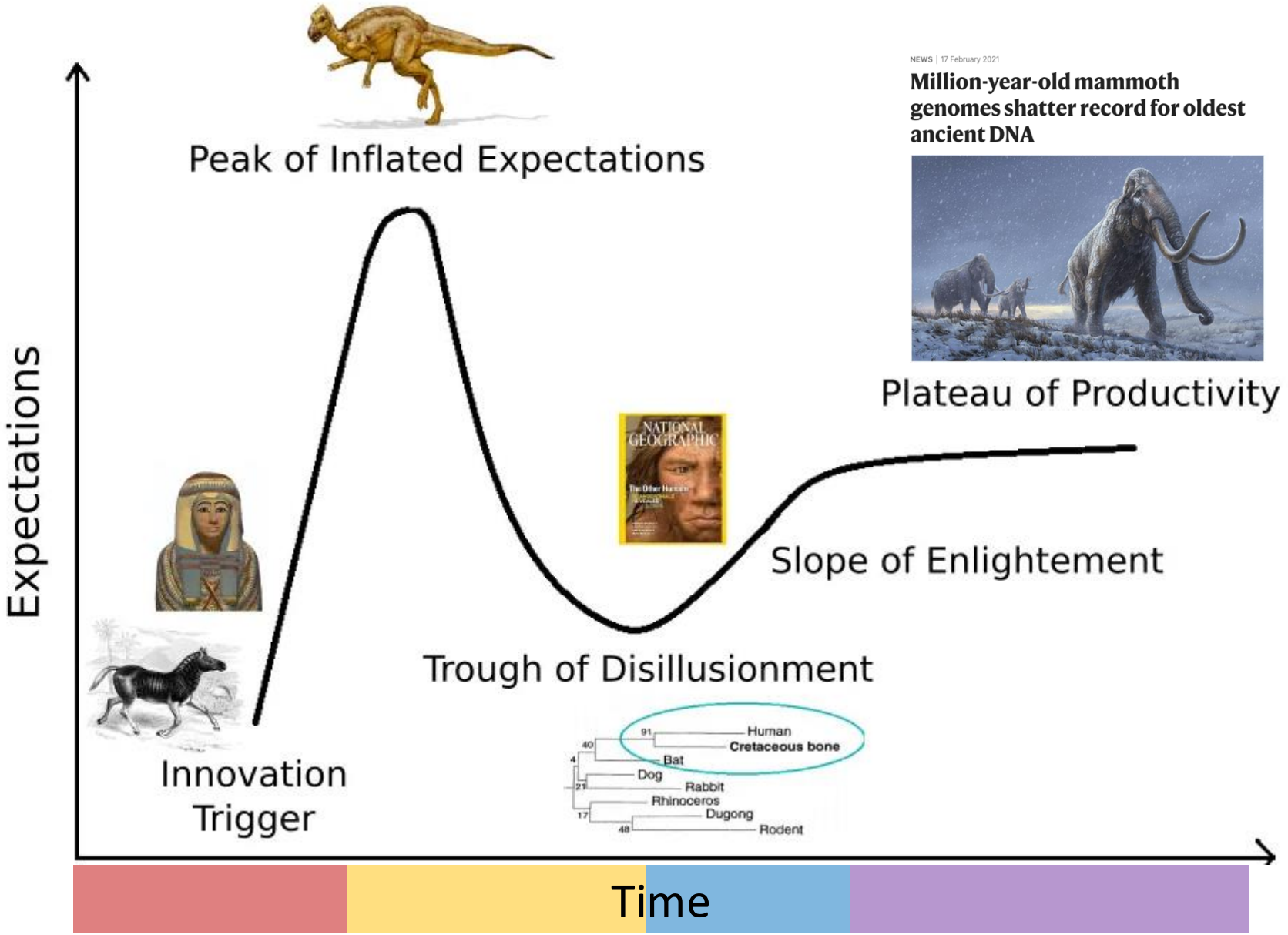
Applications of ancient DNA research



Chen and Nedoluzhko 2023 BMC Genomics

Ancient DNA is coming of age

- First ancient DNA studies:
  - ✓ Higushi et al. (1984) Nature
  - ✓ Pääbo et al. (1985) Nature
- Discredited study claiming dinosaur ancient DNA:
  - ✓ Woodward et al. (1994) Science
- First draft of the Neanderthal genome:
  - ✓ Green et al. (2010) Science
- First genome over a million years old:
  - ✓ Van der Valk et al. (2021) Nature



NEWS | 17 February 2021

Million-year-old mammoth genomes shatter record for oldest ancient DNA

Adapted from Patrícia Pečnerová 2017 The Molecular Ecologist

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Sources of ancient DNA

BONES

DENTAL CALCULUS

MAIZE COBS

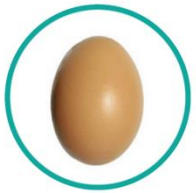
MOLLUSC SHELLS

TEETH

MUSEUM SKINS

GRAPE VINE & SEEDS

EGGSHELLS



BULK-BONE

COPROLITES

HERBARIUM

PARCHMENT

MUMMIES

RODENT MIDDEN

POLLEN

SPELEOTHEM

HAIRS

CLOTHING

WOOD

SEDIMENT CORES

Adapted from www.moleculareecologist.com/2017/04/unusual-sources-of-ancient-dna

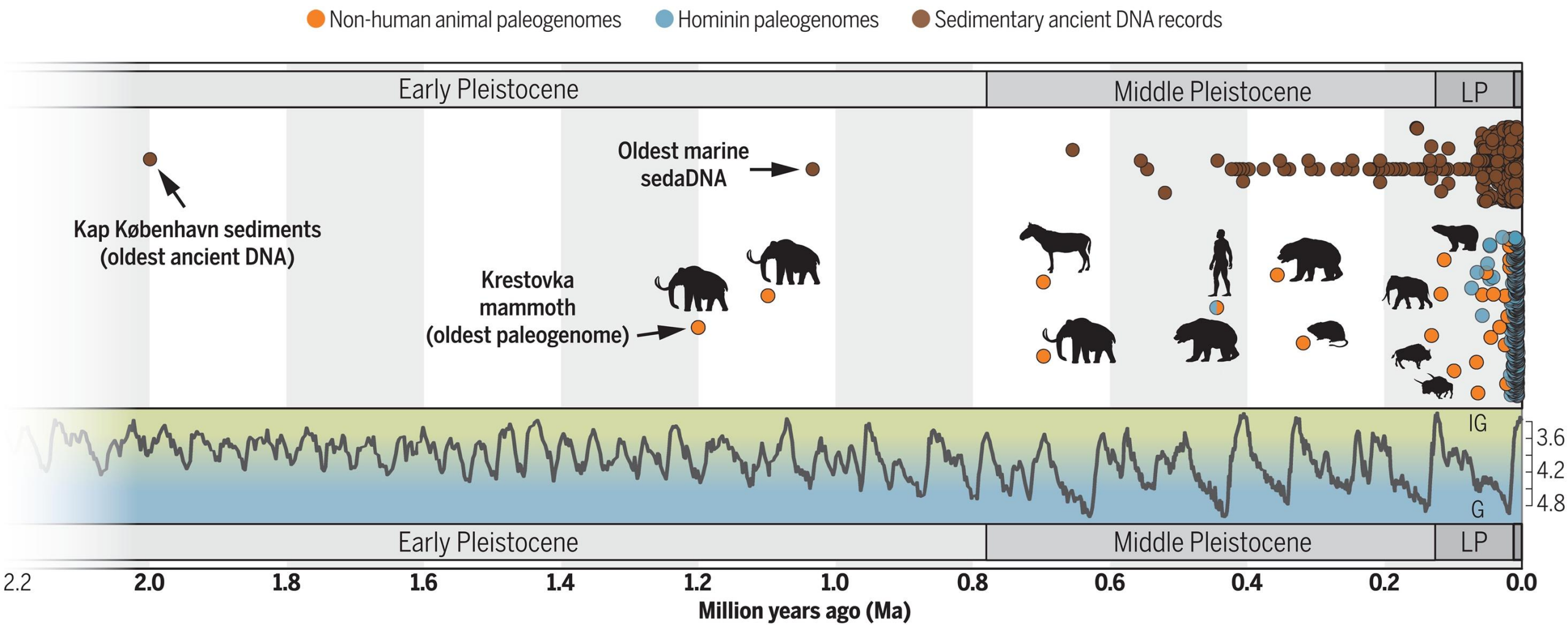
Sampling is intrinsically linked to archaeology

Post-mortem DNA decay and contamination

- DNA decay starts immediately after death due to endonucleases and microorganisms
- Over time, hydrolytic and oxidative processes will keep degrading DNA
- Favourable conditions (low temperature, anoxic, dry) help preserve DNA over thousands of years

Modified from https://www.nobelprize.org/prizes/medicine/2022/press-release/

Empirical time limit of DNA preservation



Dalén et al. 2023 Science

Empirical time limit of DNA preservation is ~1.5 million years

Wikimedia Commons

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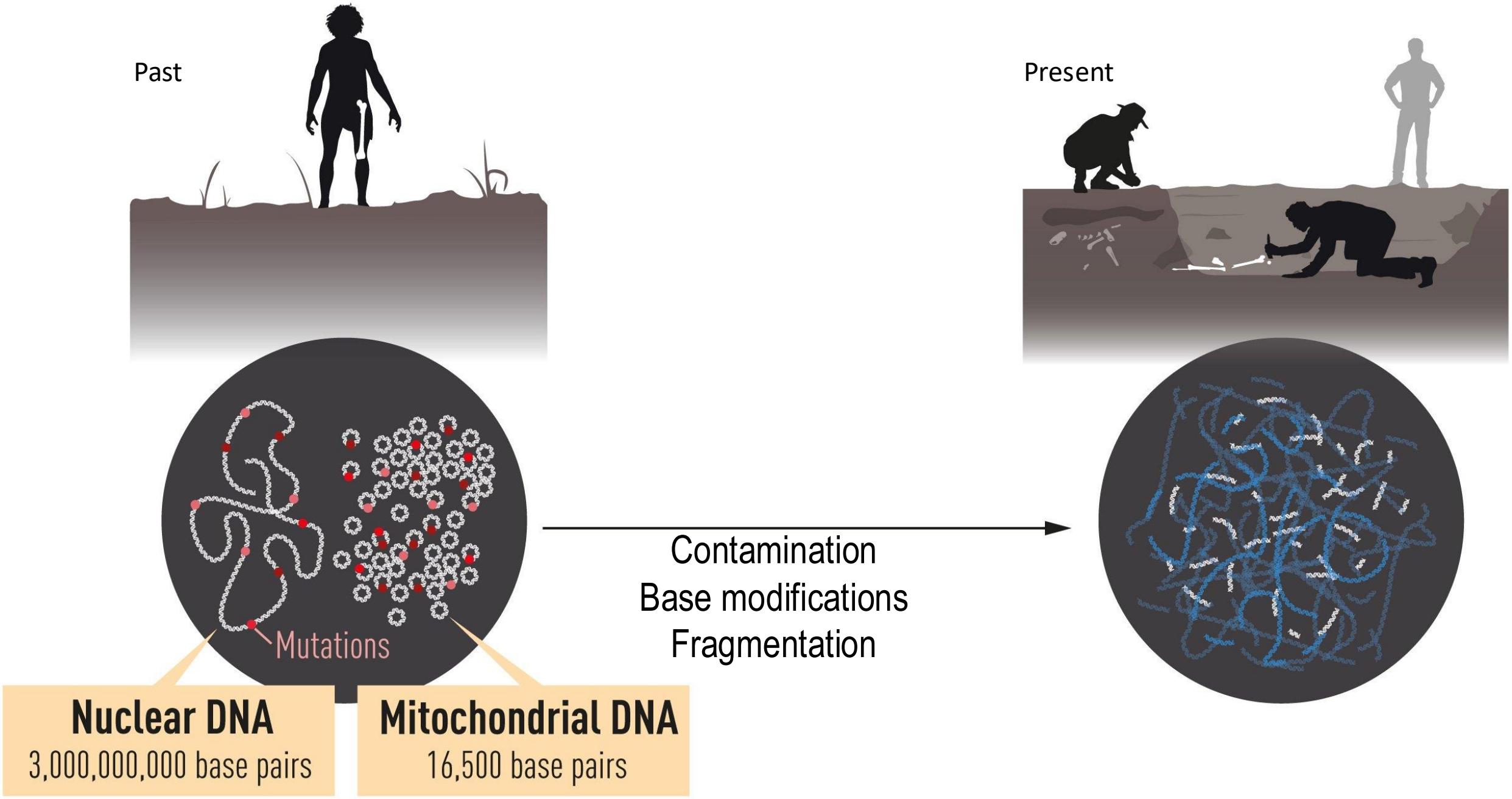
The three main properties of ancient DNA



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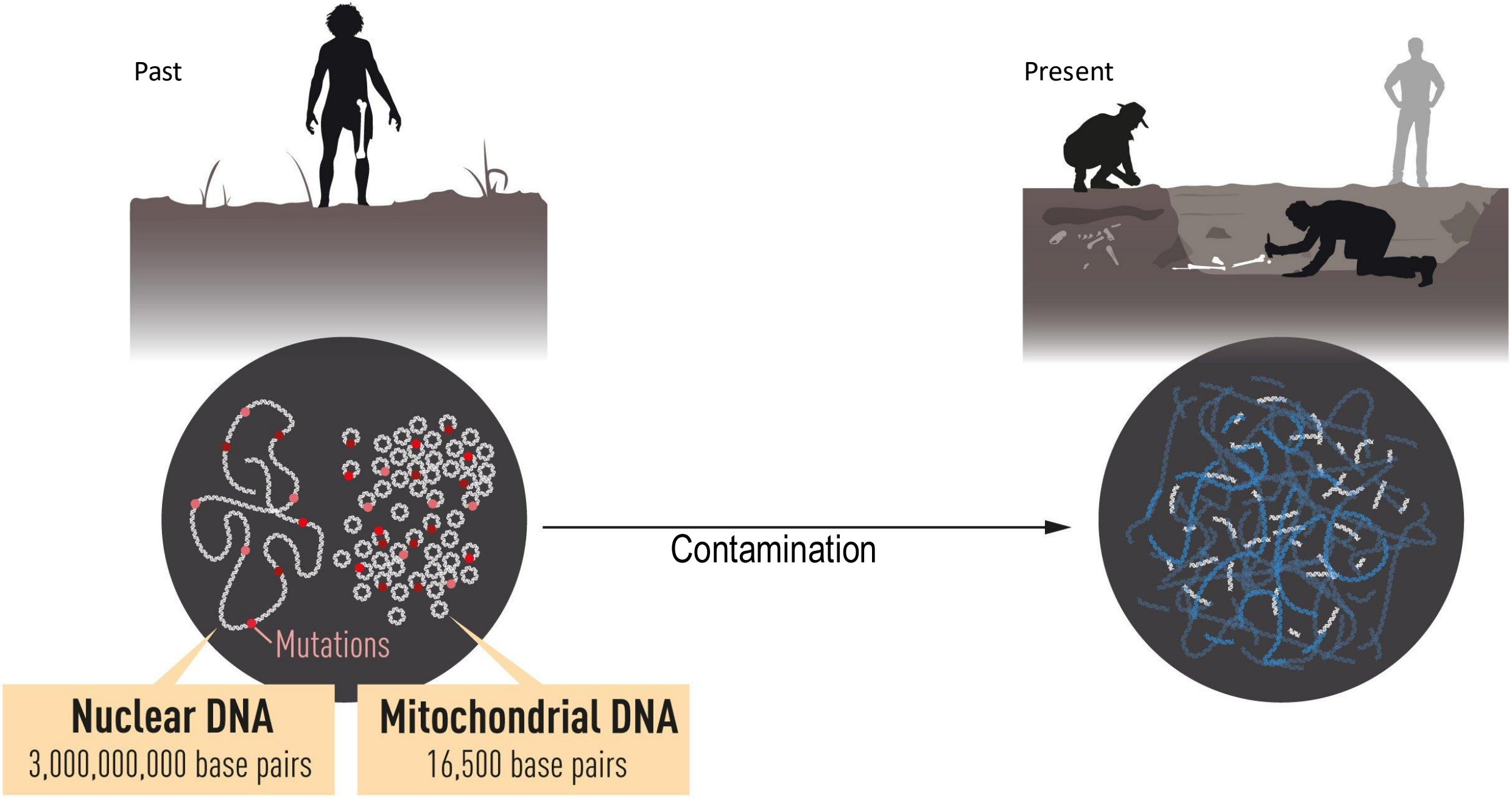
The first main property of ancient DNA: contamination



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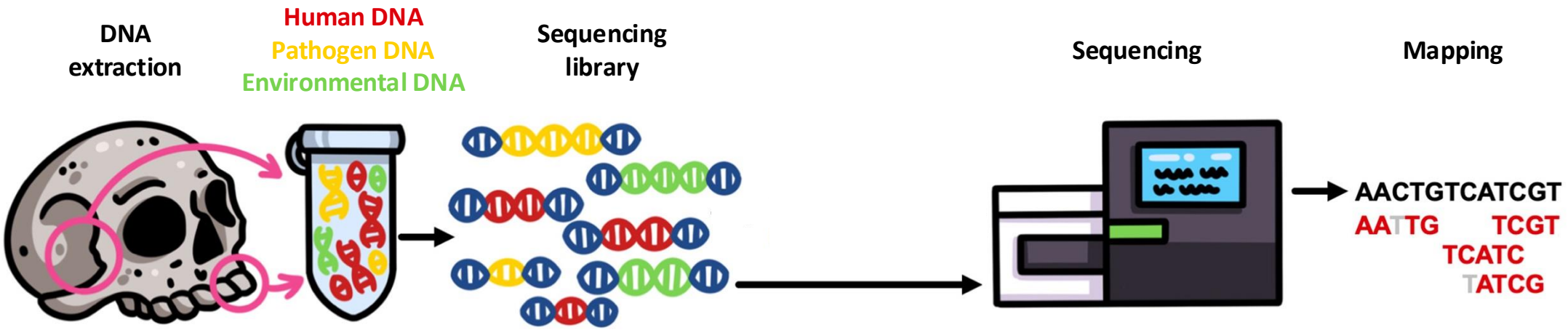
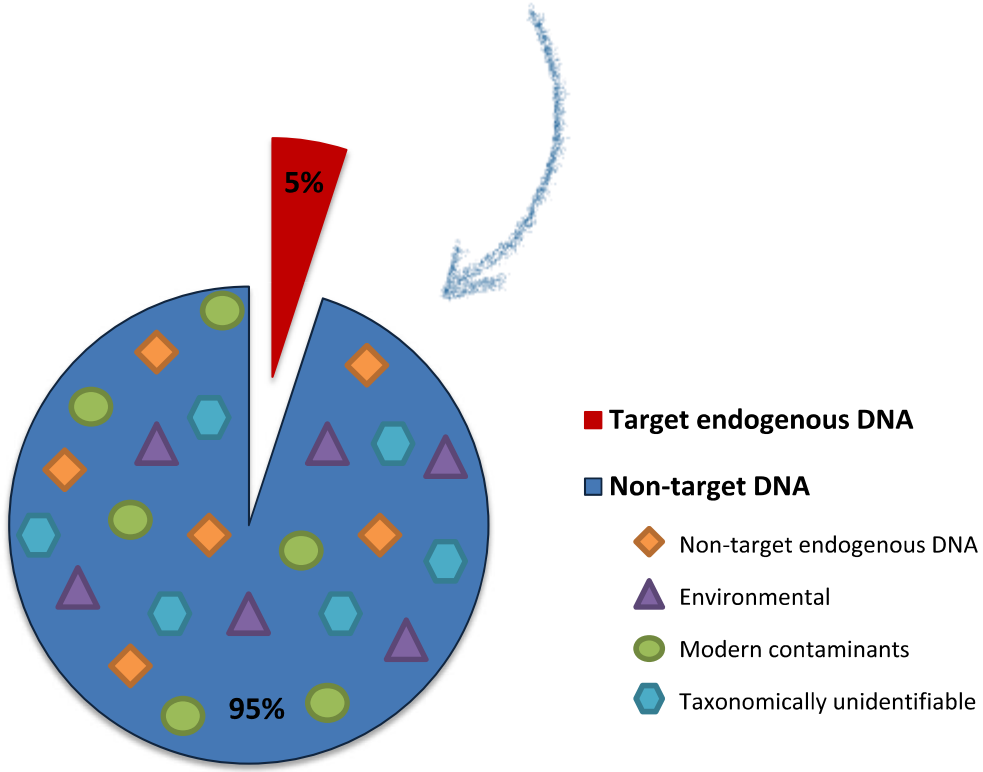
The issue of DNA contaminants from the environment





Marciniak et al 2015 J Hum Evol

- Target endogenous DNA represents a low proportions of all DNA molecules present in the extract
- Contaminant DNA comes mostly from the environment where the samples were found
- Contaminant DNA is mostly microbial, but can also come from plants and animals
- Present-day human DNA is problematic when studying ancient human DNA

Modified from Childebayeva & Zavala 2023 iScience

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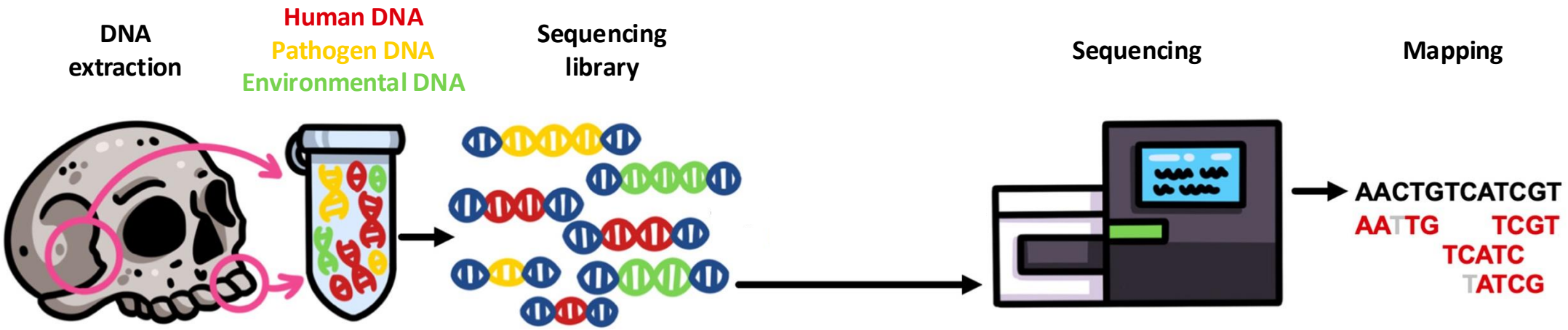

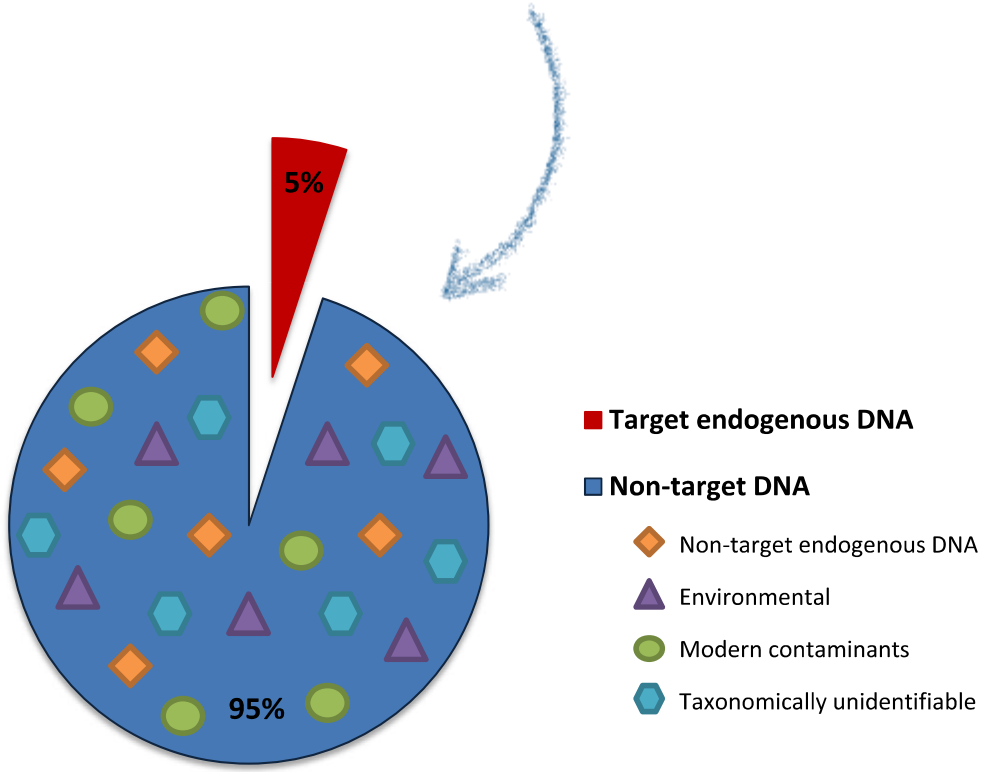

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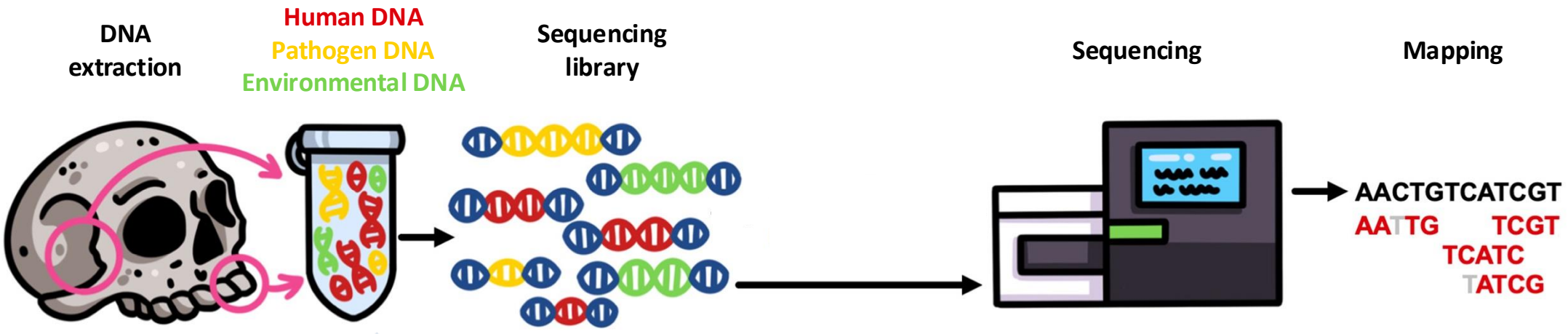

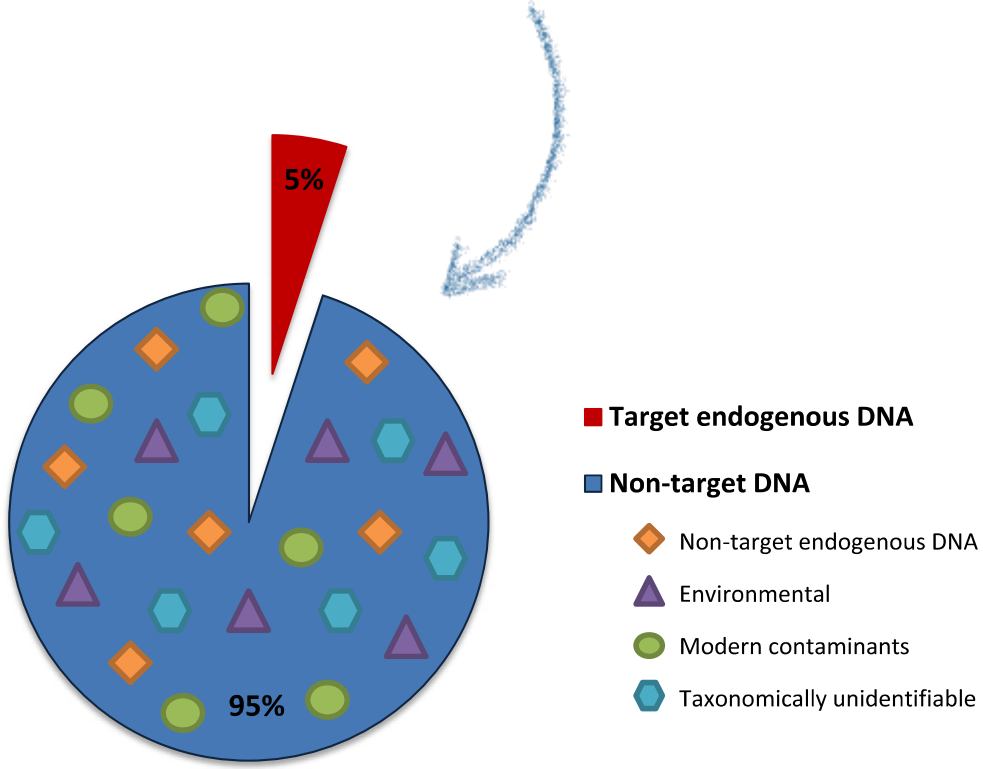

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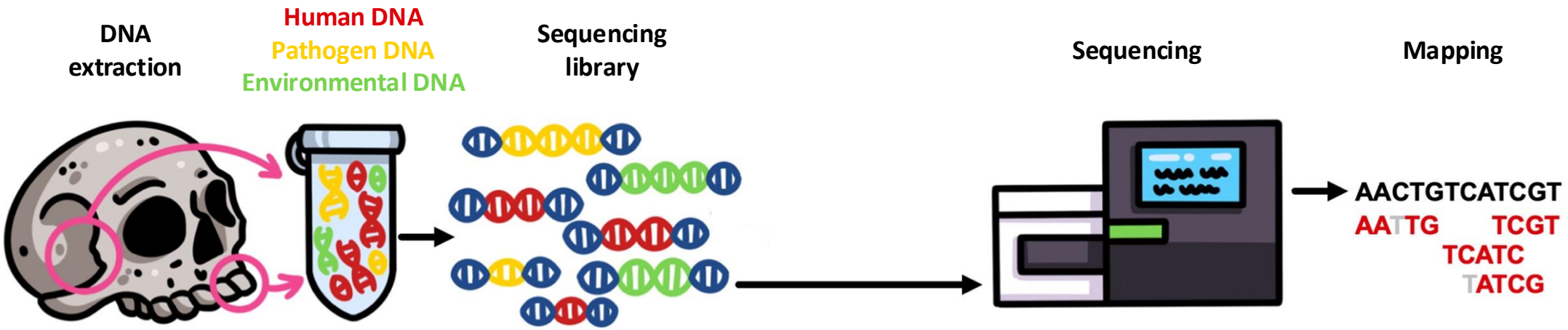

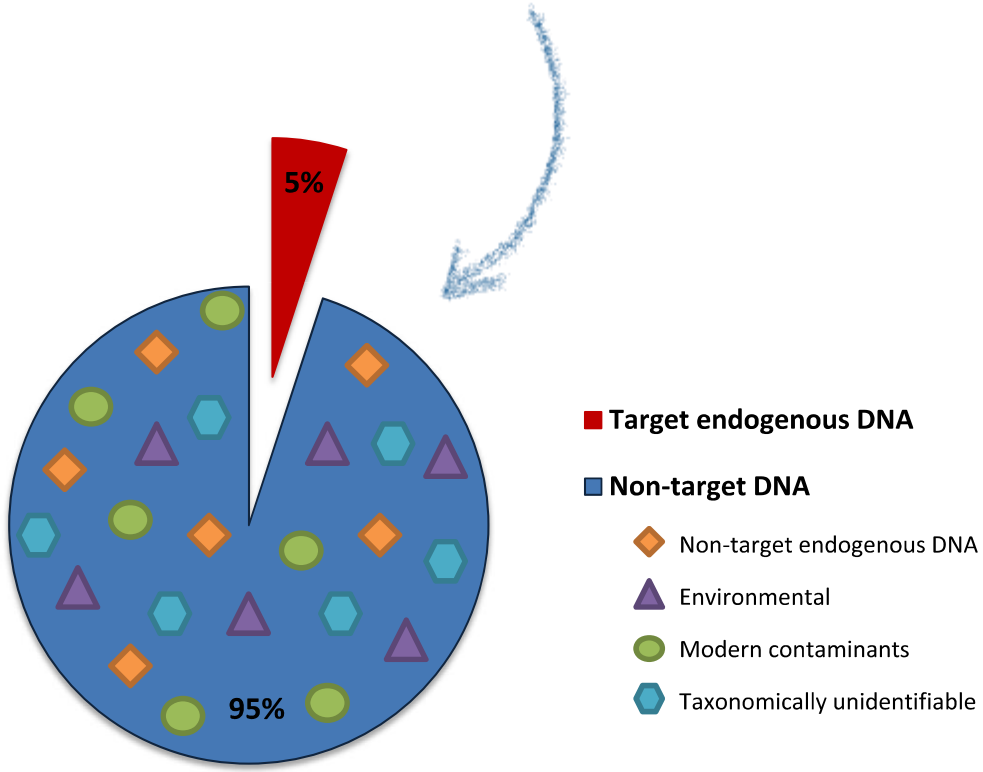

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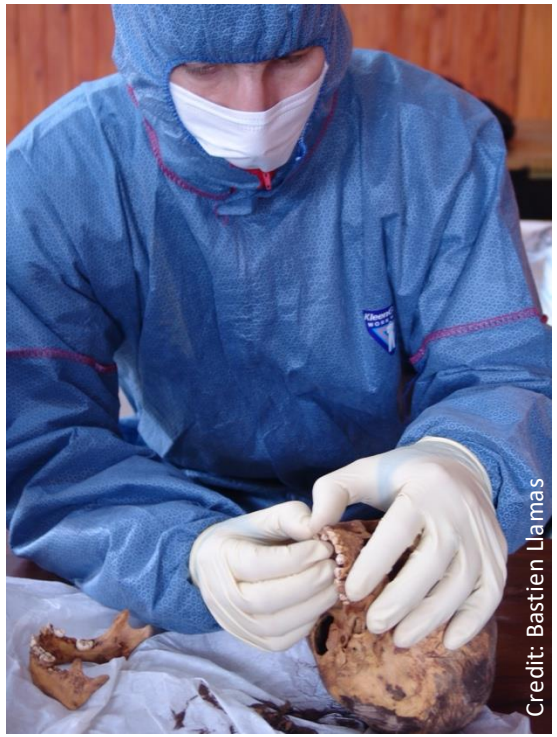
Protect the sample from yourself



PPE in the field and in the lab

The Australian Centre for Ancient DNA: a low-DNA environment

Google Maps

Credit: Jeremy Austin

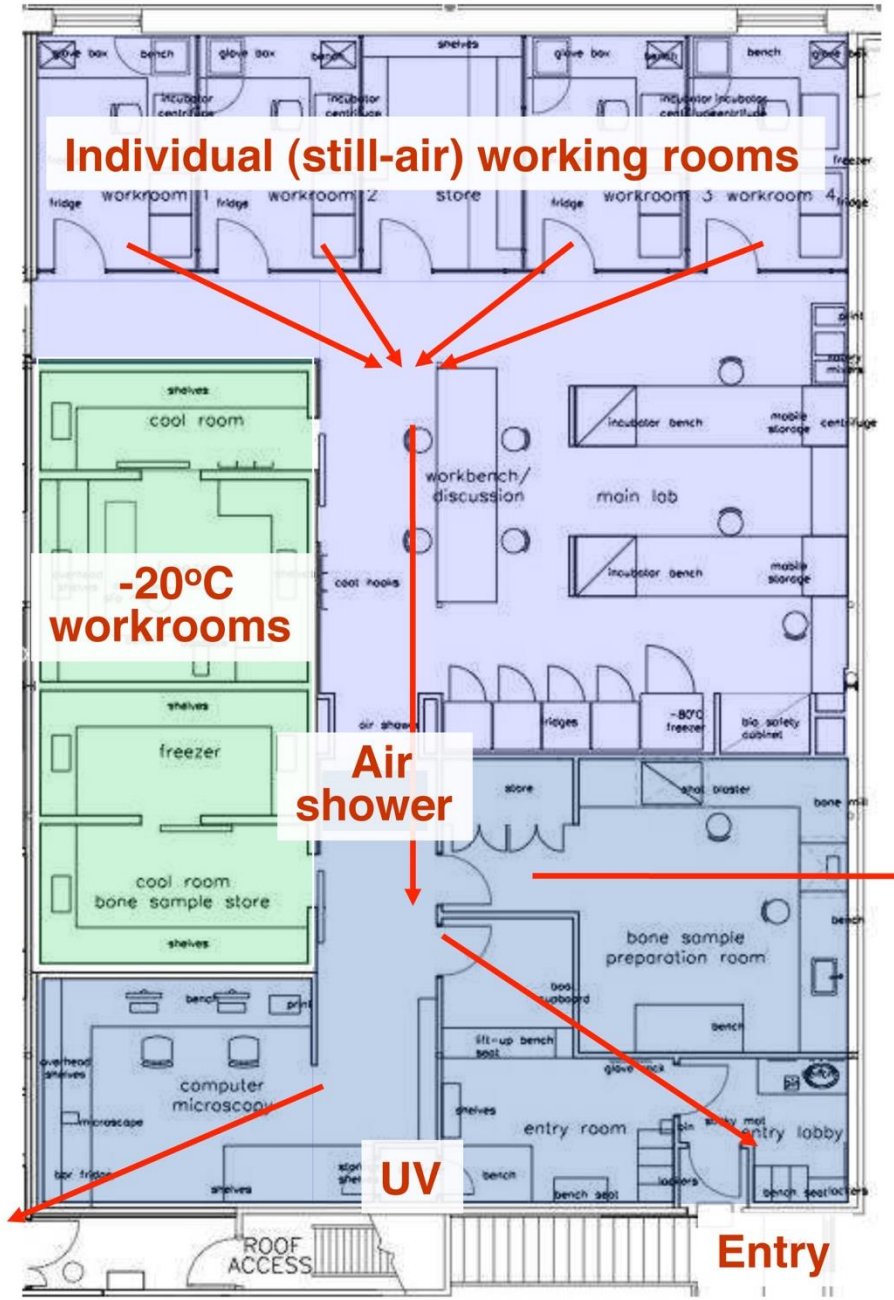
Specialist laboratory facilities

- Clean rooms physically isolated from sources of DNA contaminants:
  - No amplification of DNA
  - Positive air pressure

- Clean rooms dedicated to specific tasks:
  - Sample storage
  - Sample preparation
  - DNA extraction
  - Molecular biology (pre-amplification)

- UV and bleach treatment of work surfaces
- DNA-free reagents and consumables
- PPE and strict protocols

Credit: Julien Soubrier



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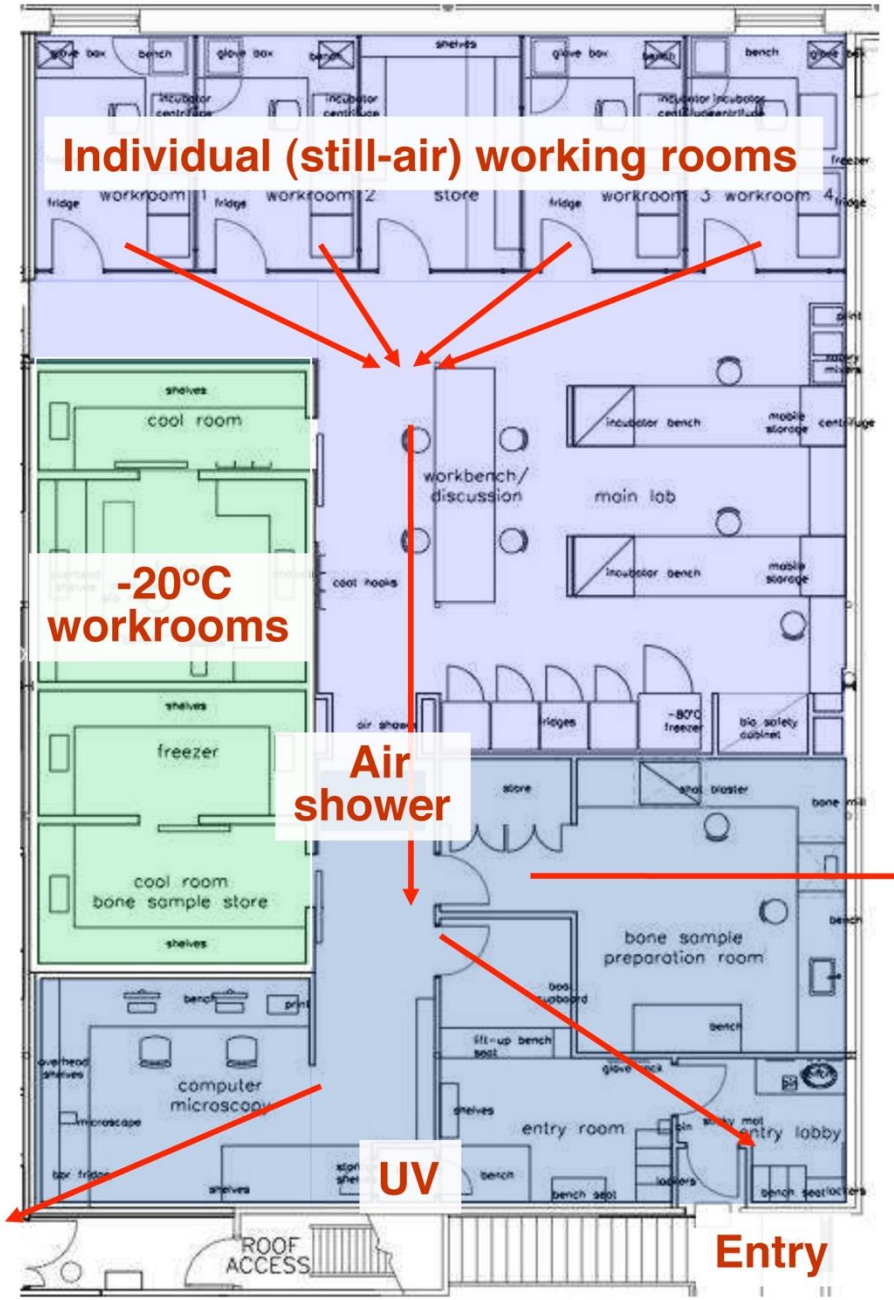
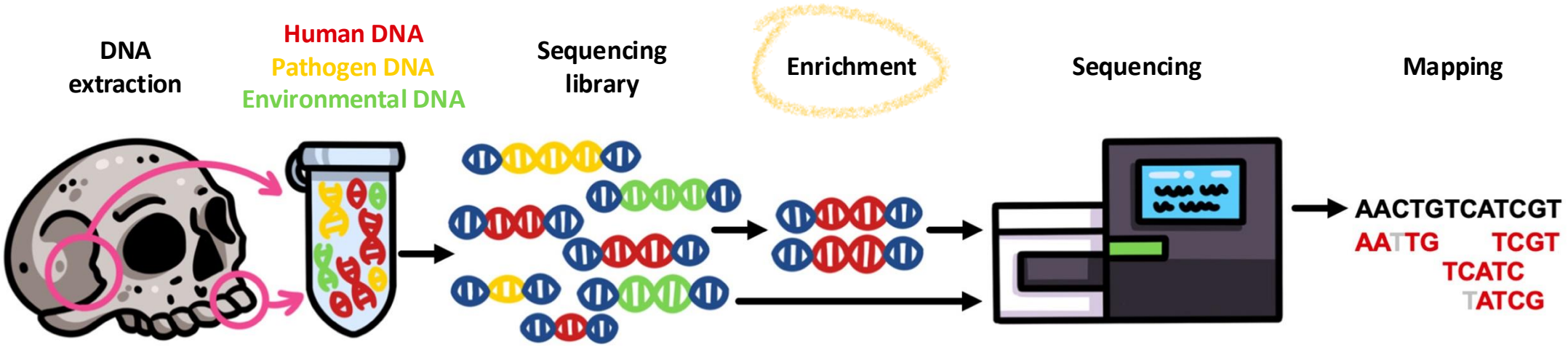


Enrichment of target DNA



- Enrich target endogenous DNA using hybridisation to DNA or RNA baits

- Target endogenous DNA can be:
  - The whole genome
  - A chromosome
  - The mitochondrial genome
  - All the genes (the exome)
  - Genome-wide variable positions (Single Nucleotide polymorphisms—SNPs)
  - chrY SNPs

Modified from Childebayeva & Zavala 2023 iScience

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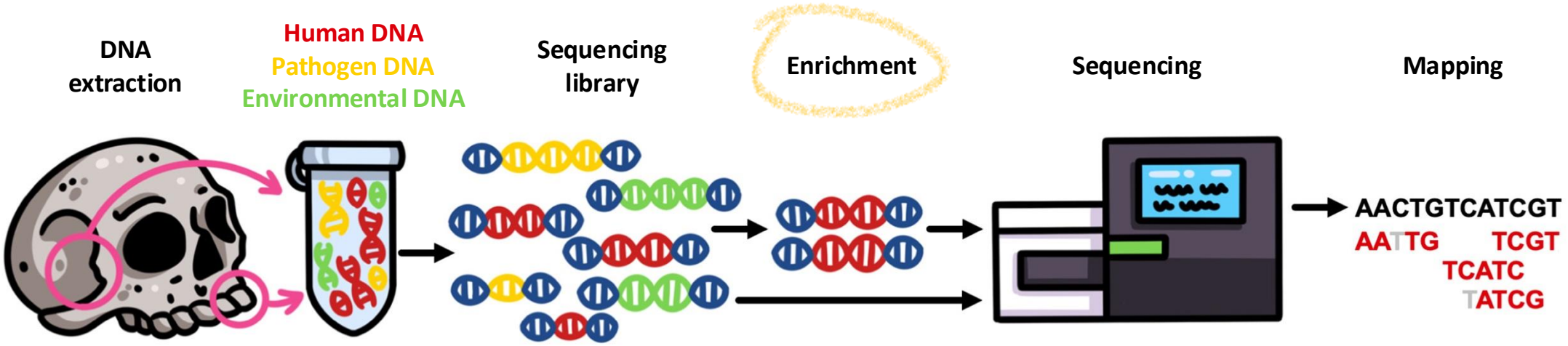

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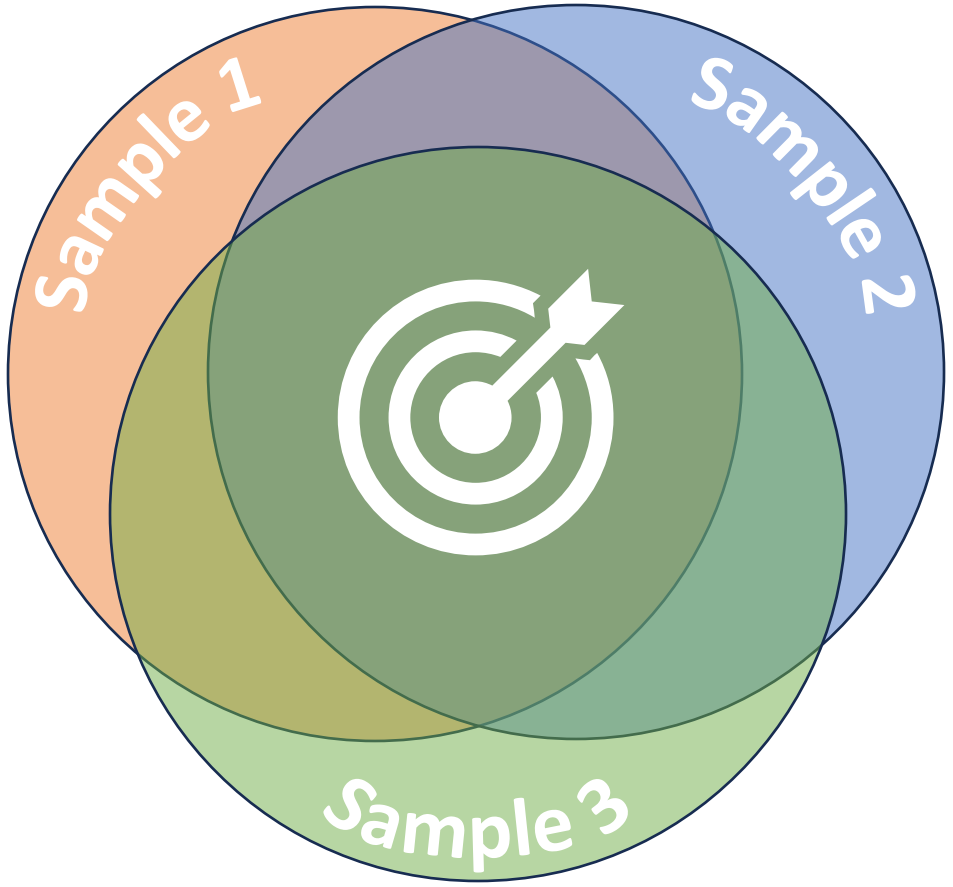
Modified from Childebayeva & Zavala 2023 iScience

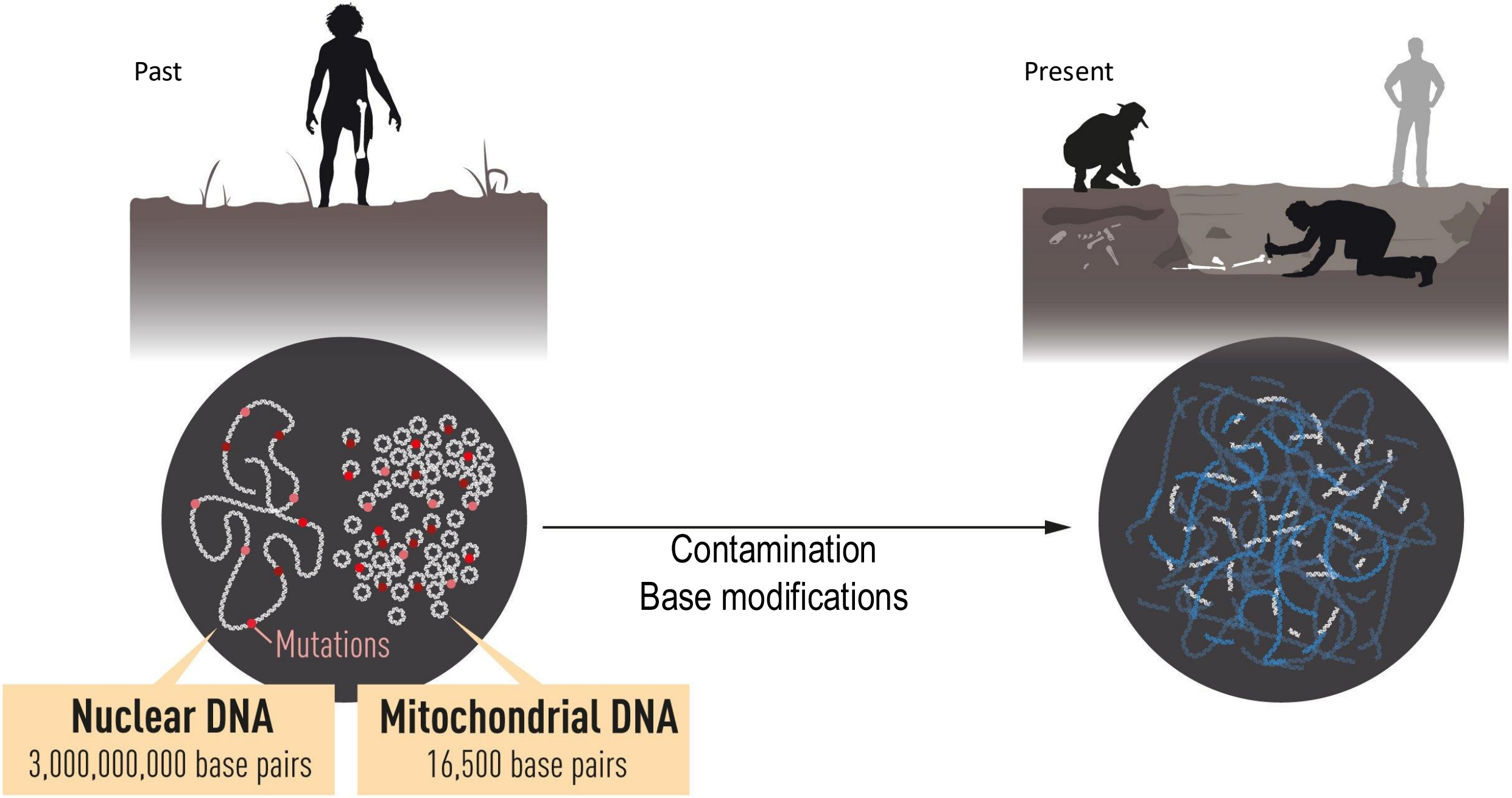
Benefits of target DNA enrichment

Benefits include:

- Increase the proportion of endogenous DNA in the sequencing data
- Increase the proportion of endogenous DNA common between samples
- Decreased sequencing costs



The second main property of ancient DNA: base modifications



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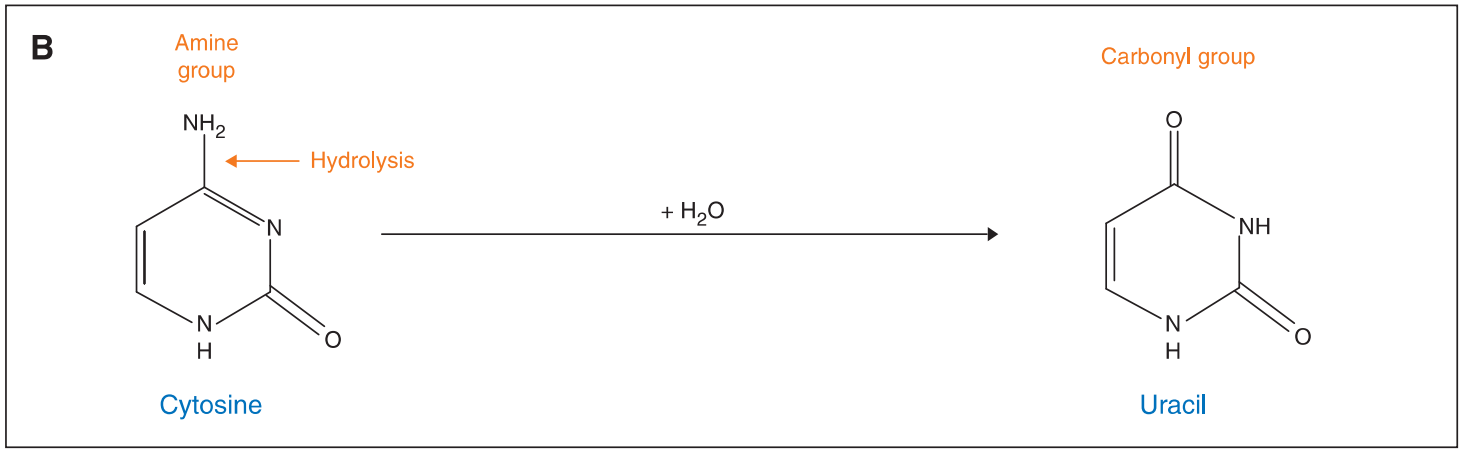
Ancient DNA damage: cytosine deamination

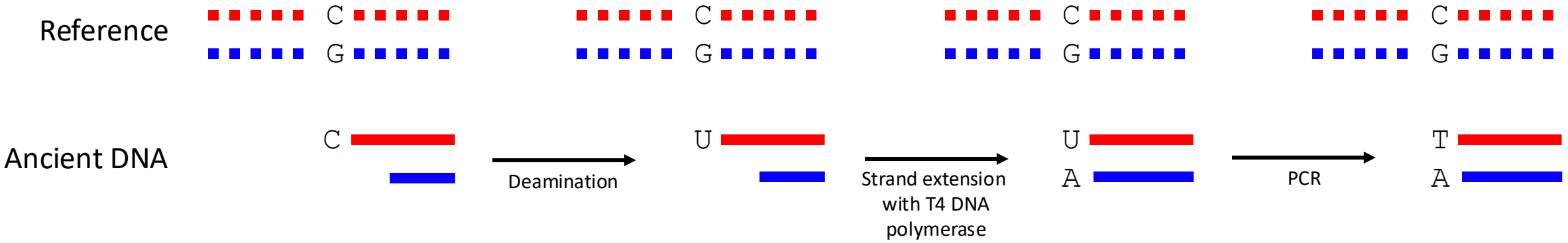


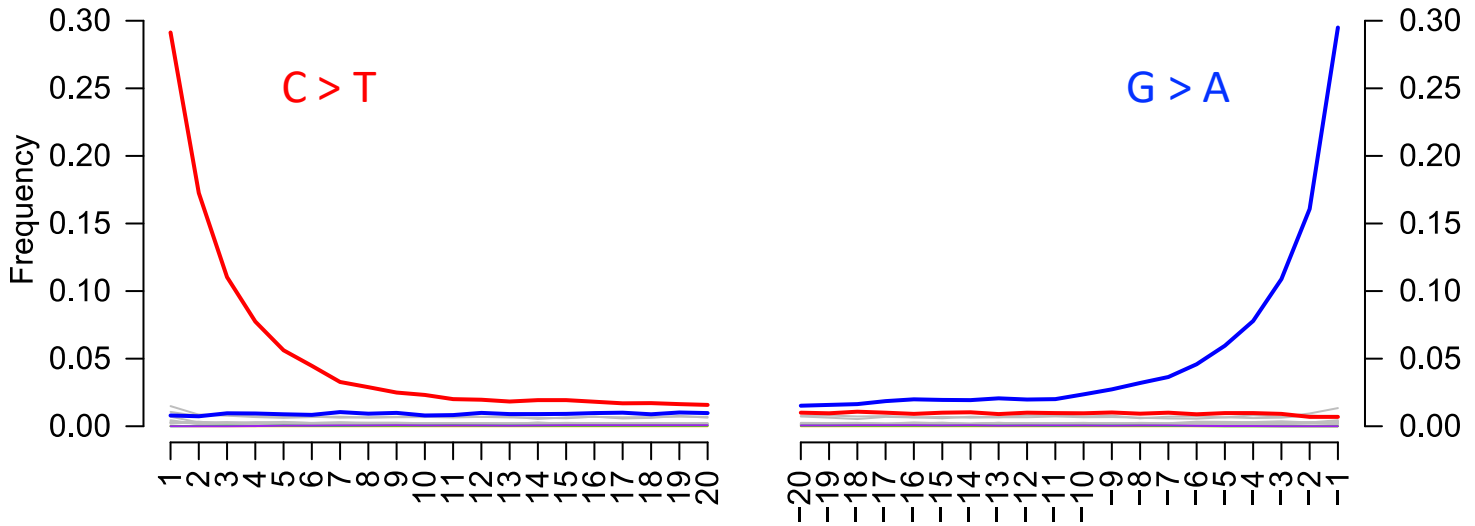
Dabney et al 2013 Cold Spring Harbor perspectives in biology

- Deamination of cytosine to uracil is the major mechanism leading to miscoding lesions in ancient DNA
- If not dealt with experimentally or bioinformatically, miscoding lesions can lead to false positive results

Cytosine deamination accumulates at ancient molecules ends

DNA polymerases will incorporate an A across from the U, and in turn a T across from the A, causing apparent G to A and C to T substitutions





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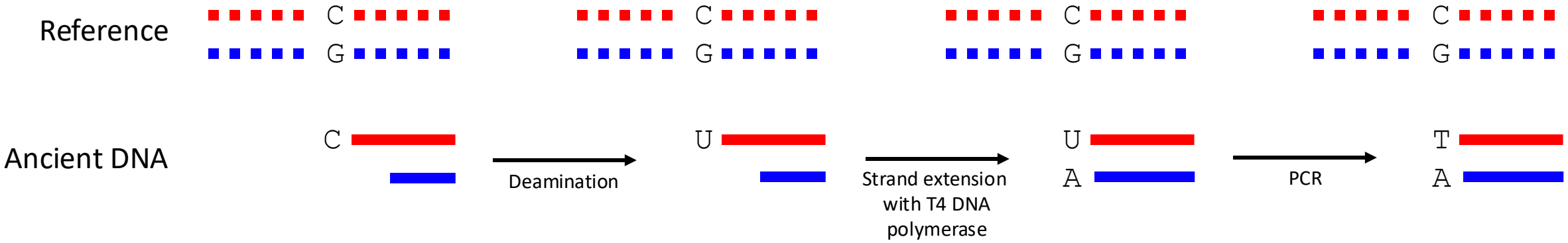
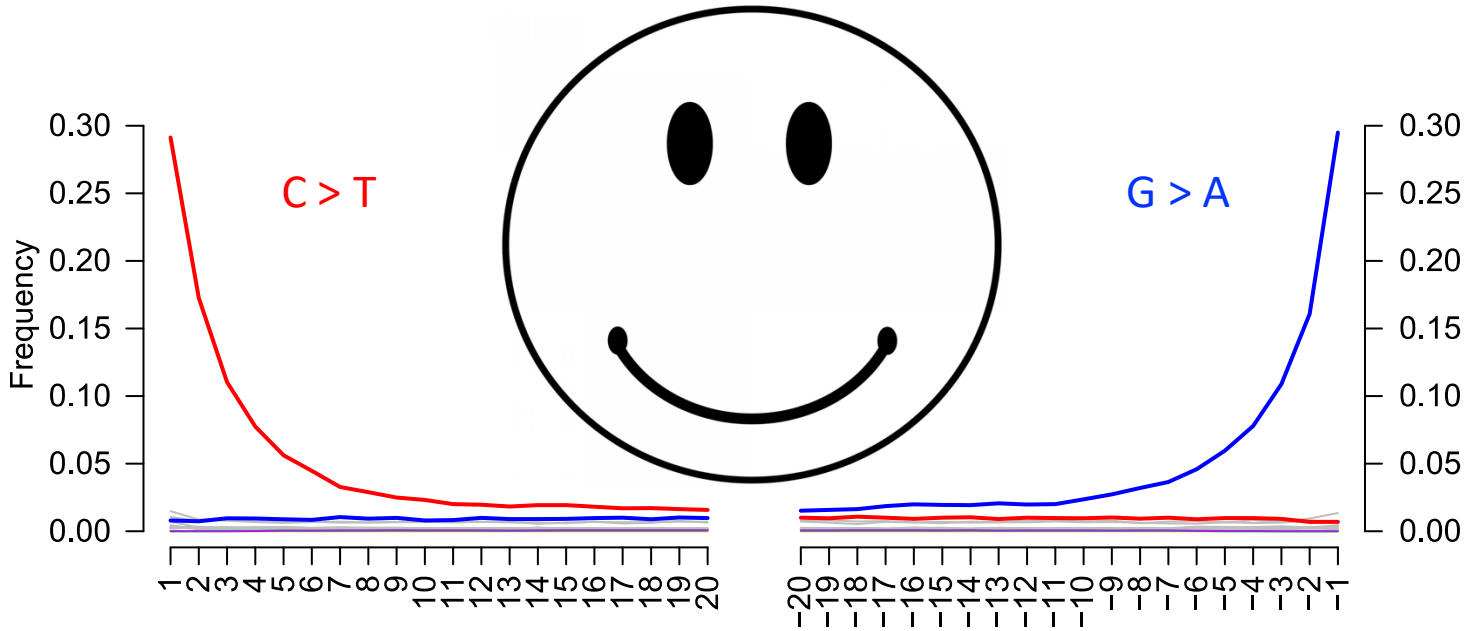
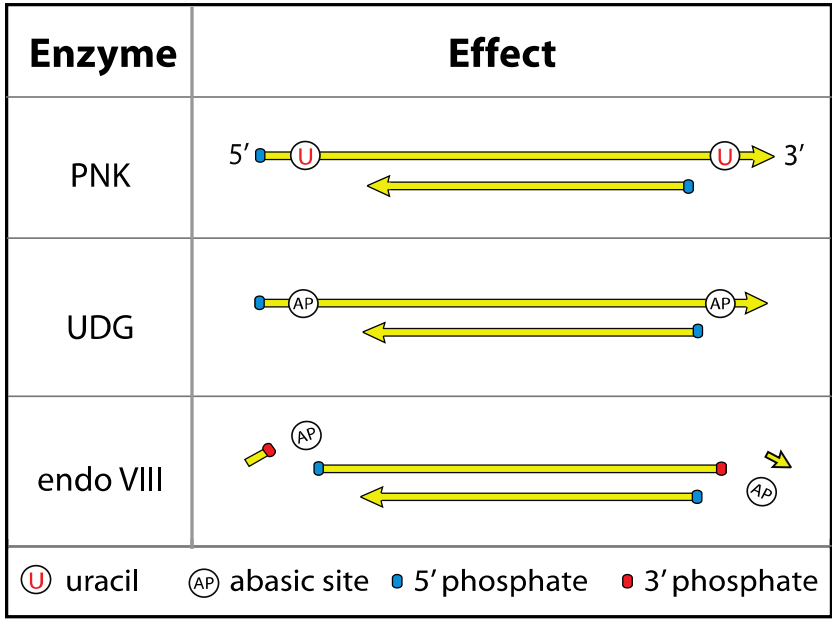




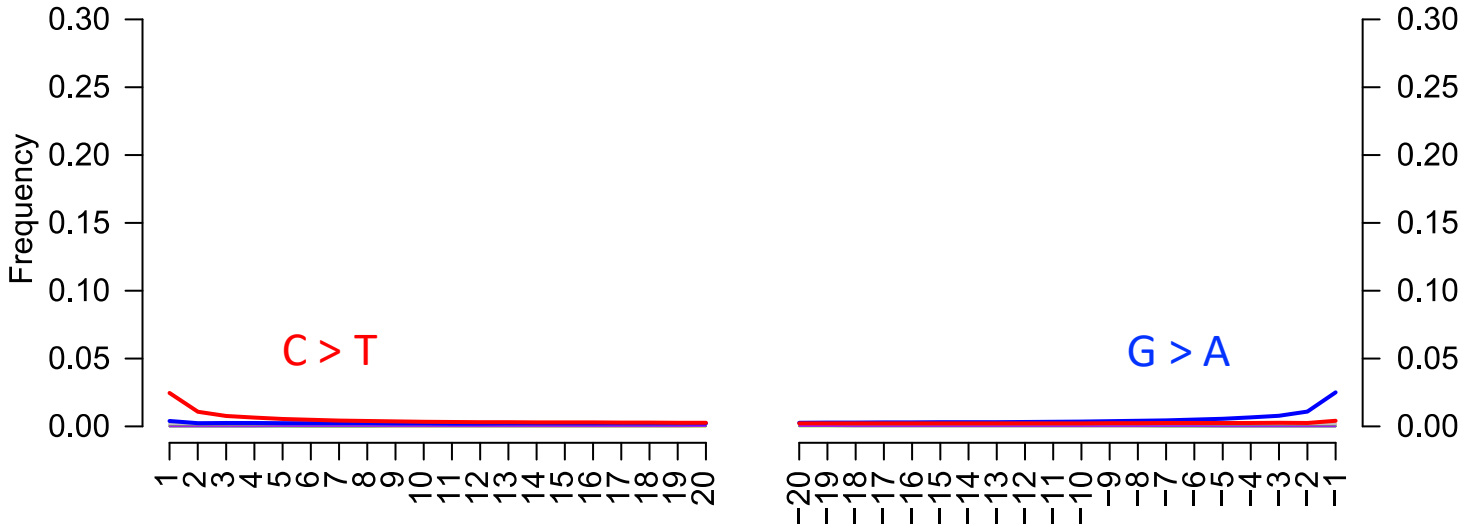
Uracils can be removed experimentally

DNA can be “repaired” before preparing sequencing libraries by:

- Removing the uracil base with UDG (Uracil-DNA Glycosylase), leaving an abasic site
- Cleaving the DNA backbone at the 3’ and 5’ sides of the abasic site with endonuclease VIII



Adapted from Briggs et al. 2010 Nucleic Acids Research



Consequences of DNA repair:

- No accumulation of C-to-T and G-to-A substitutions at the DNA fragment ends
- Shorter DNA fragments

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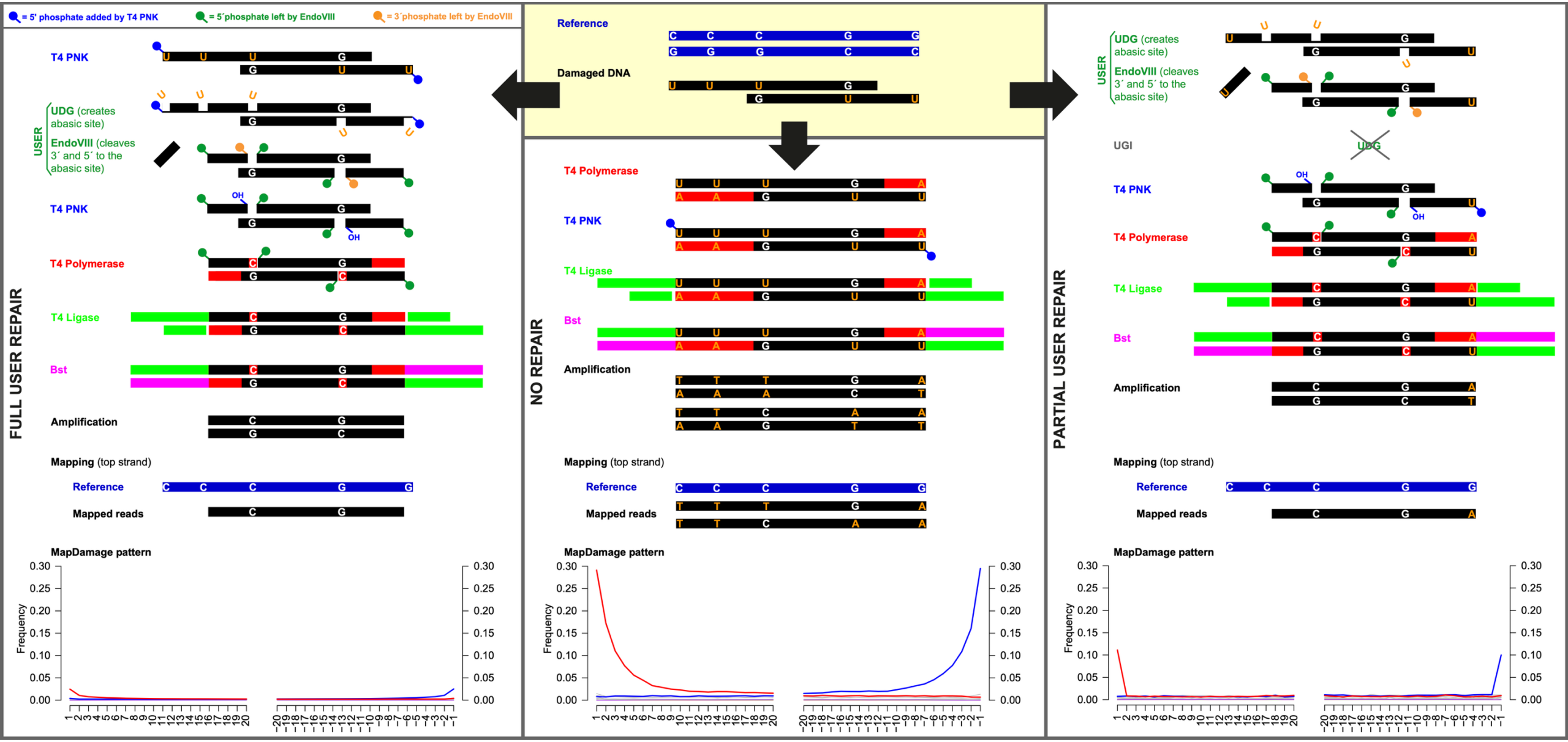
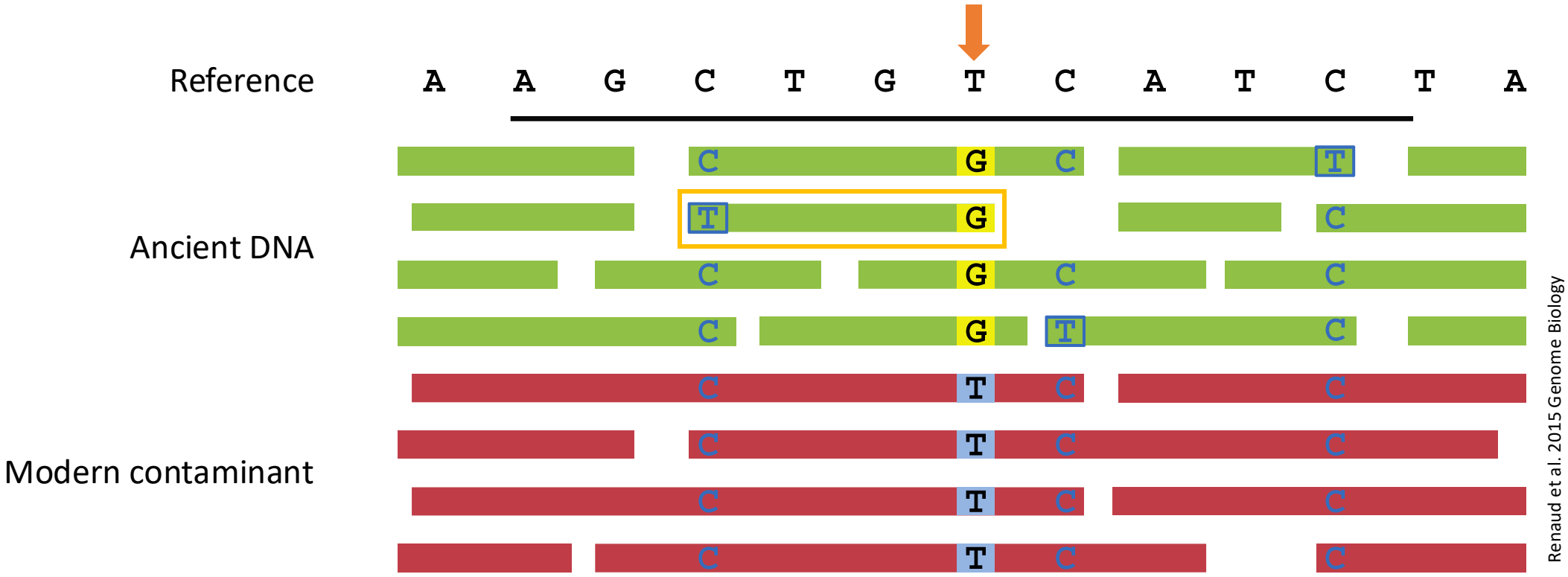


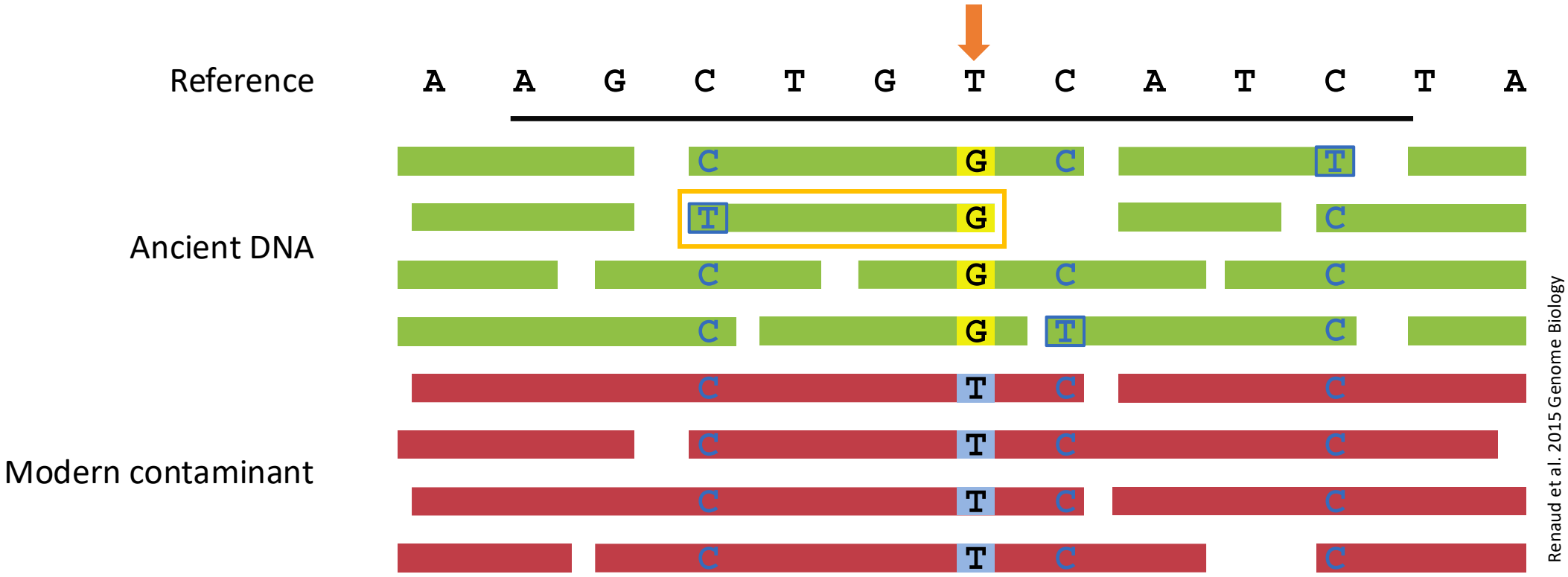
Contamination estimation from haploid DNA



In data mapped to haploid genomes (mitochondrial DNA in both sexes or X chromosome in males):

1. Identify heteroplasmic positions (here G/T, orange down arrow)
2. Identify ancient DNA fragments that carry deaminated cytosines near the ends of the DNA fragments (T in blue squares)
3. The ancient allele is linked to cytosine deamination in some ancient DNA fragments (fragment in the orange box). Estimate contamination from fragments that do not contain the ancient allele

Contamination estimation from haploid DNA



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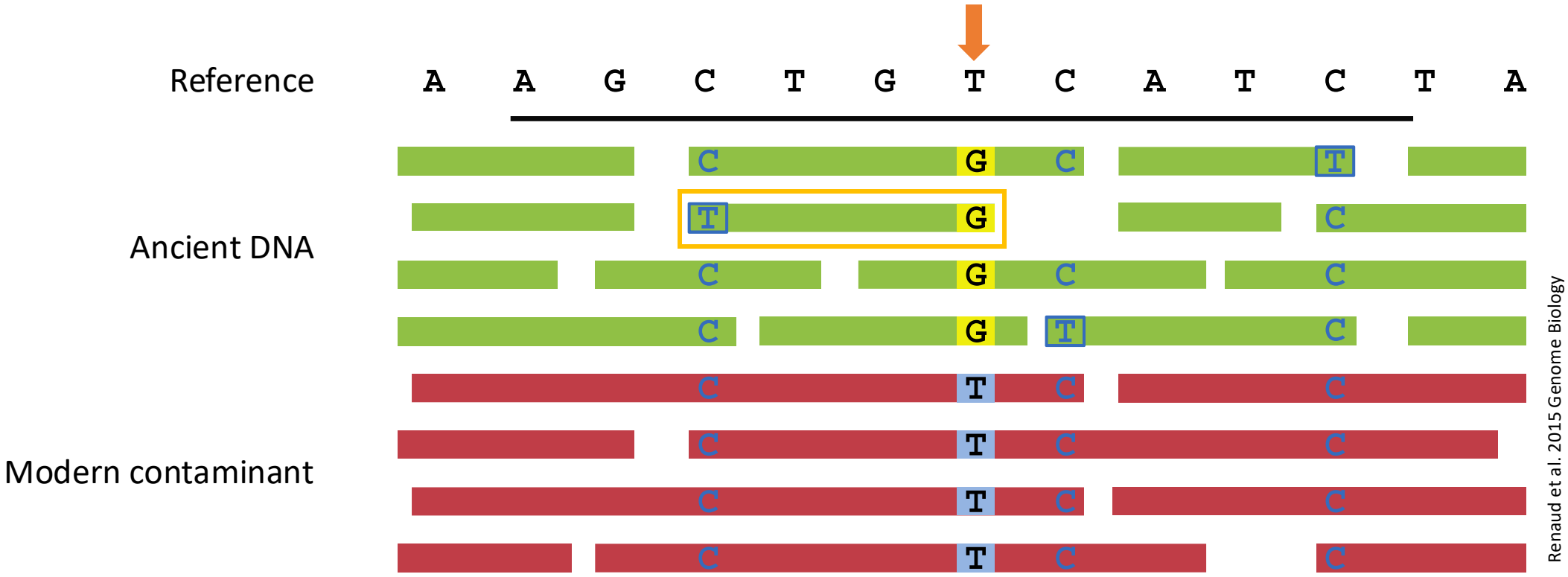

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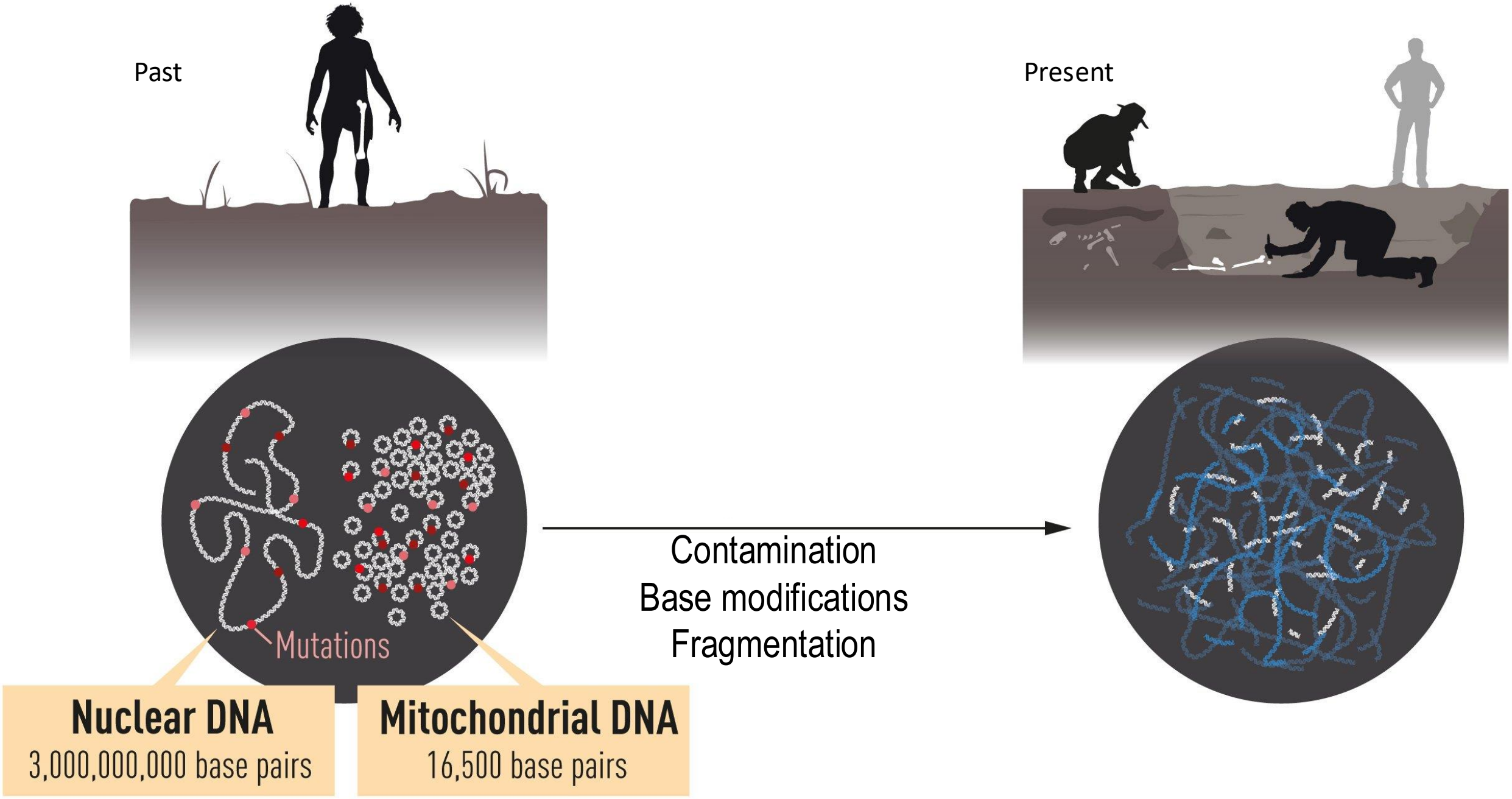
The third main property of ancient DNA: fragmentation



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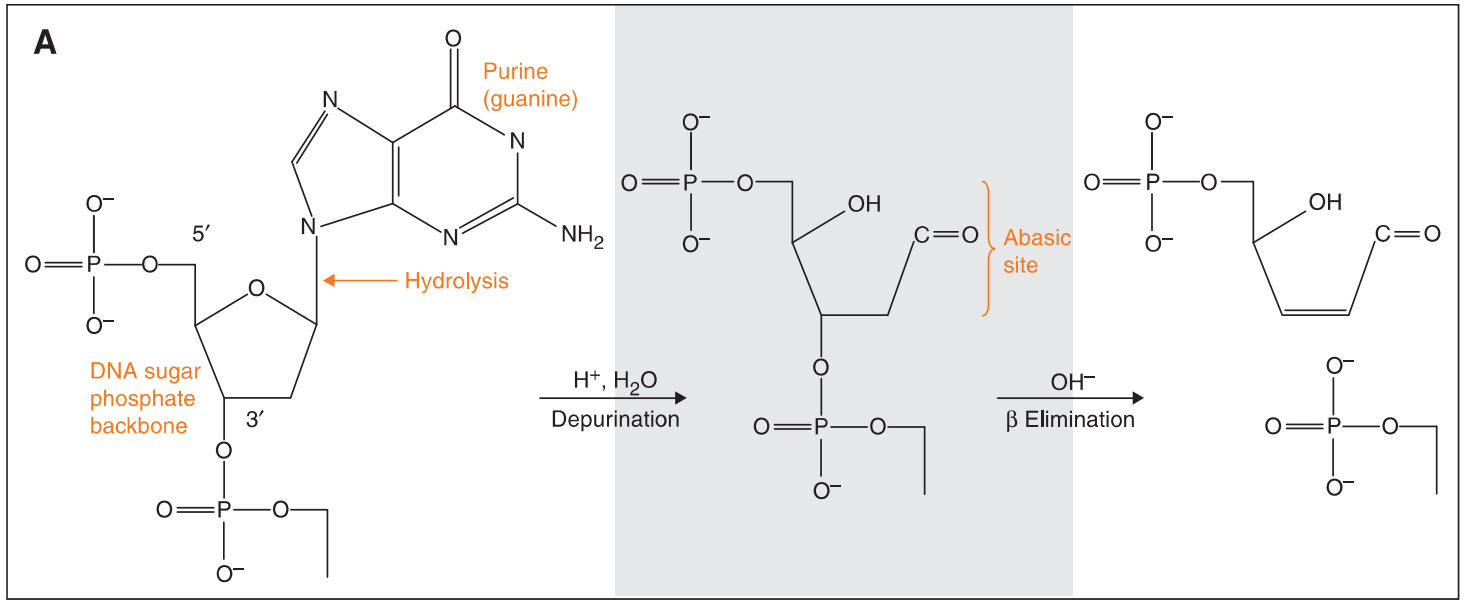
Ancient DNA fragmentation



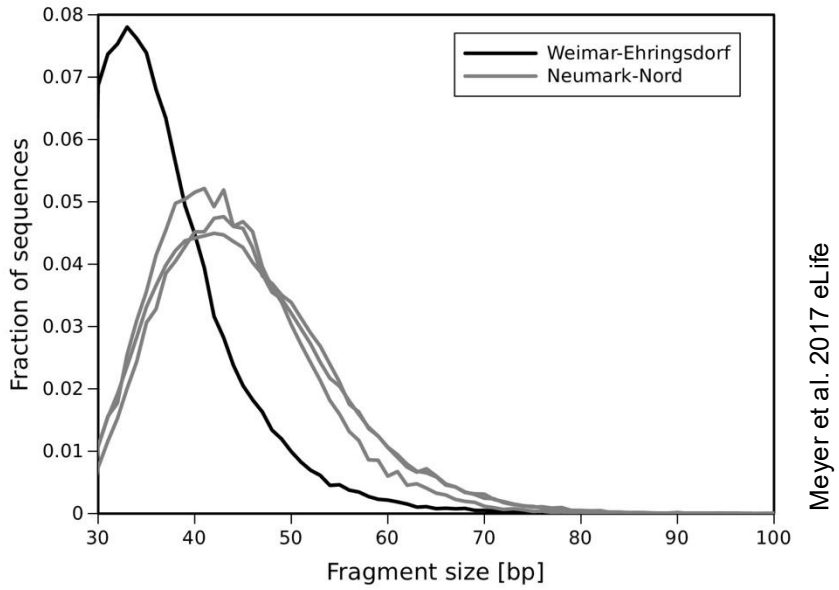
Dabney et al 2013 Cold Spring Harbor perspectives in biology

Steps to DNA fragmentation:

- Hydrolytic depurination (N-glycosyl bond between sugar and adenine or guanine residue is cleaved) results in an abasic site
- DNA strand fragmented through β elimination, leaving 3'-aldehydic and 5'-phosphate ends

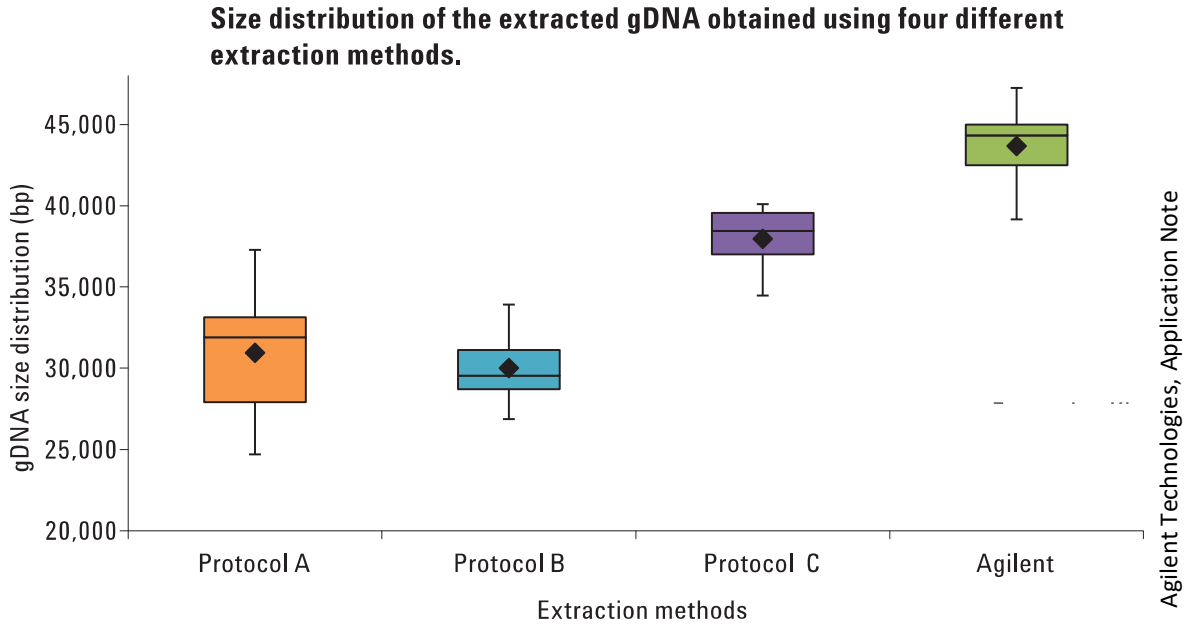
Ancient DNA fragmentation

Ancient DNA:
Few tens of base pairs



Meyer et al. 2017 eLife

DNA from fresh samples:
Several thousands of base pairs



Agilent Technologies, Application Note

- Extensive fragmentation (typically < 100 bp)
- Ideal for high-throughput short-read sequencing (HTS)
- DNA extraction protocols suitable for ultra-short DNA fragments

So, what is so special about ancient DNA?

- PROPERTY: Ancient DNA is heavily fragmented

  EVIDENCE: Distribution of ancient DNA fragments length is skewed towards short fragments

- PROPERTY : ancient DNA is contaminated with exogenous DNA

  EVIDENCE: Only a small fraction of the raw sequencing data aligns to the reference genome

- PROPERTY : Cytosines are deaminated into uracils

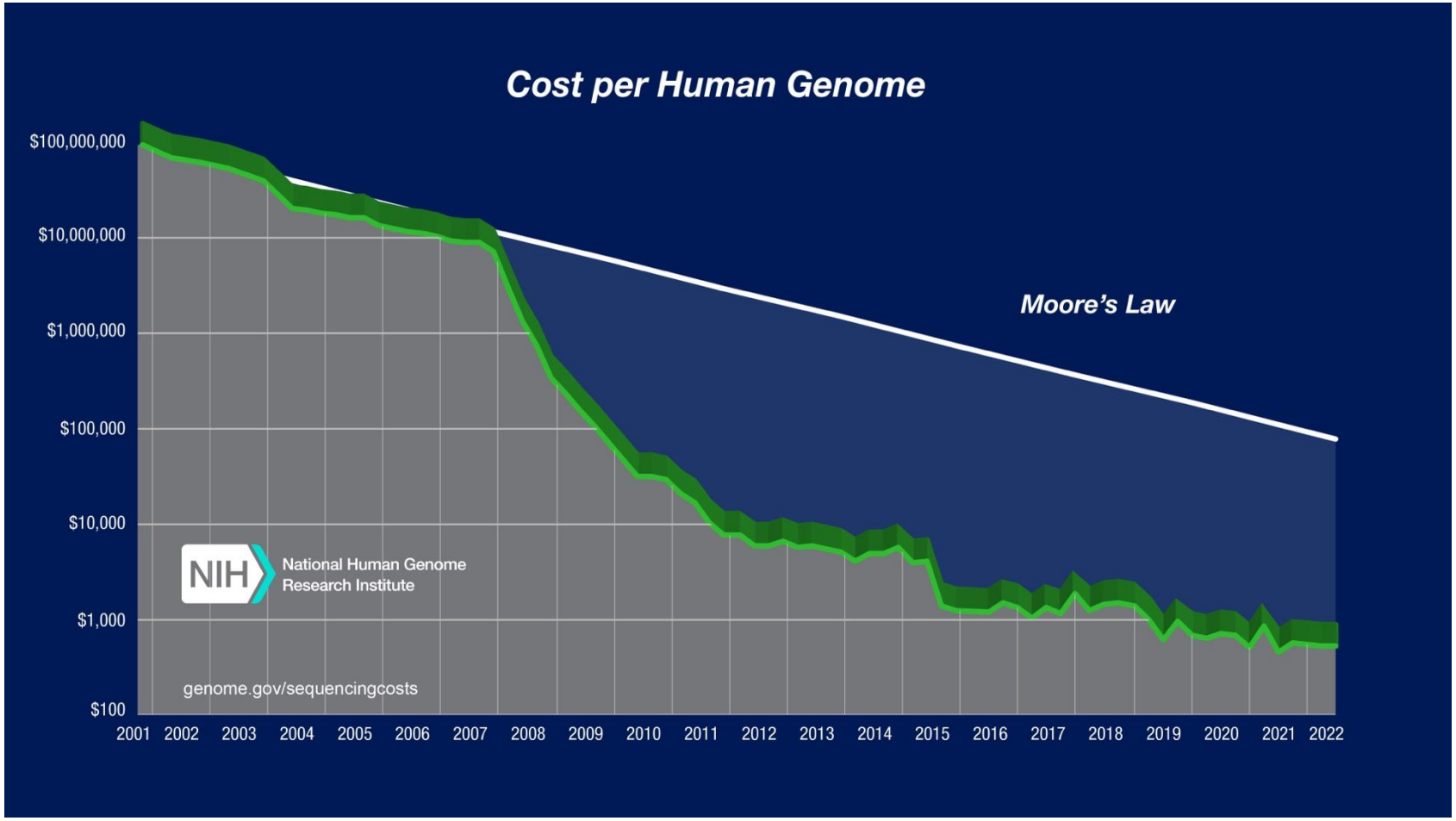
  EVIDENCE: Accumulation of C-to-T and complementary A-to-G substitutions at the ends of sequencing reads if DNA is not repaired with UDG and Endo VIII

Lecture overview

- Introduction to ancient DNA
- Sources of ancient DNA and post-mortem DNA decay
- Properties of ancient DNA
- Ancient DNA analysis

High-throughput sequencing (HTS)

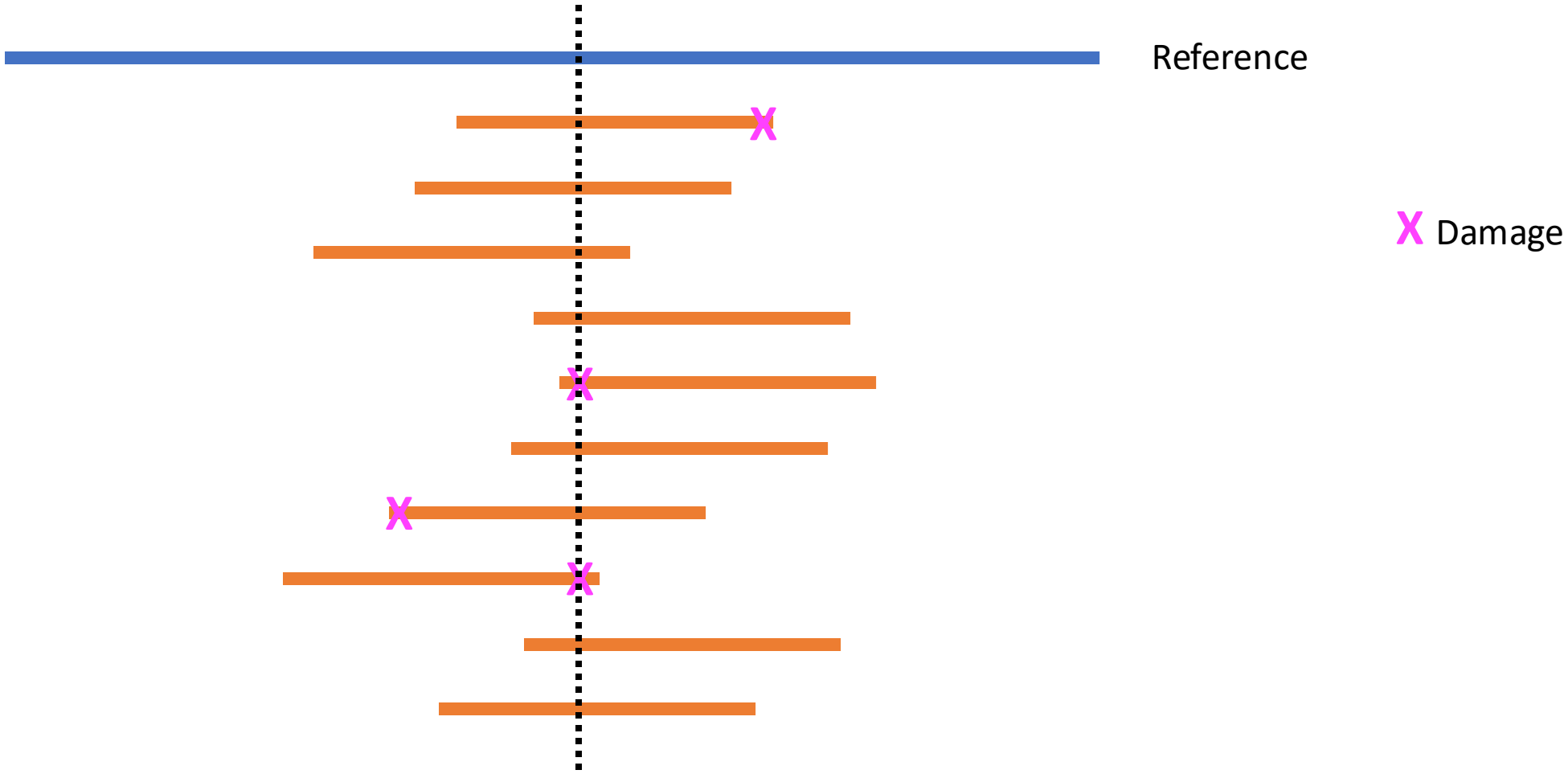
- Short-read sequencers generate DNA sequences ≤ 150 nucleotides
- Significant and sustained decrease in cost from mid-2000s



Ancient DNA and HTS: the perfect match

- HTS generate short DNA sequences
- Sequencing of virtually all molecules in an ancient DNA extract
- Independent replication for each genomic site

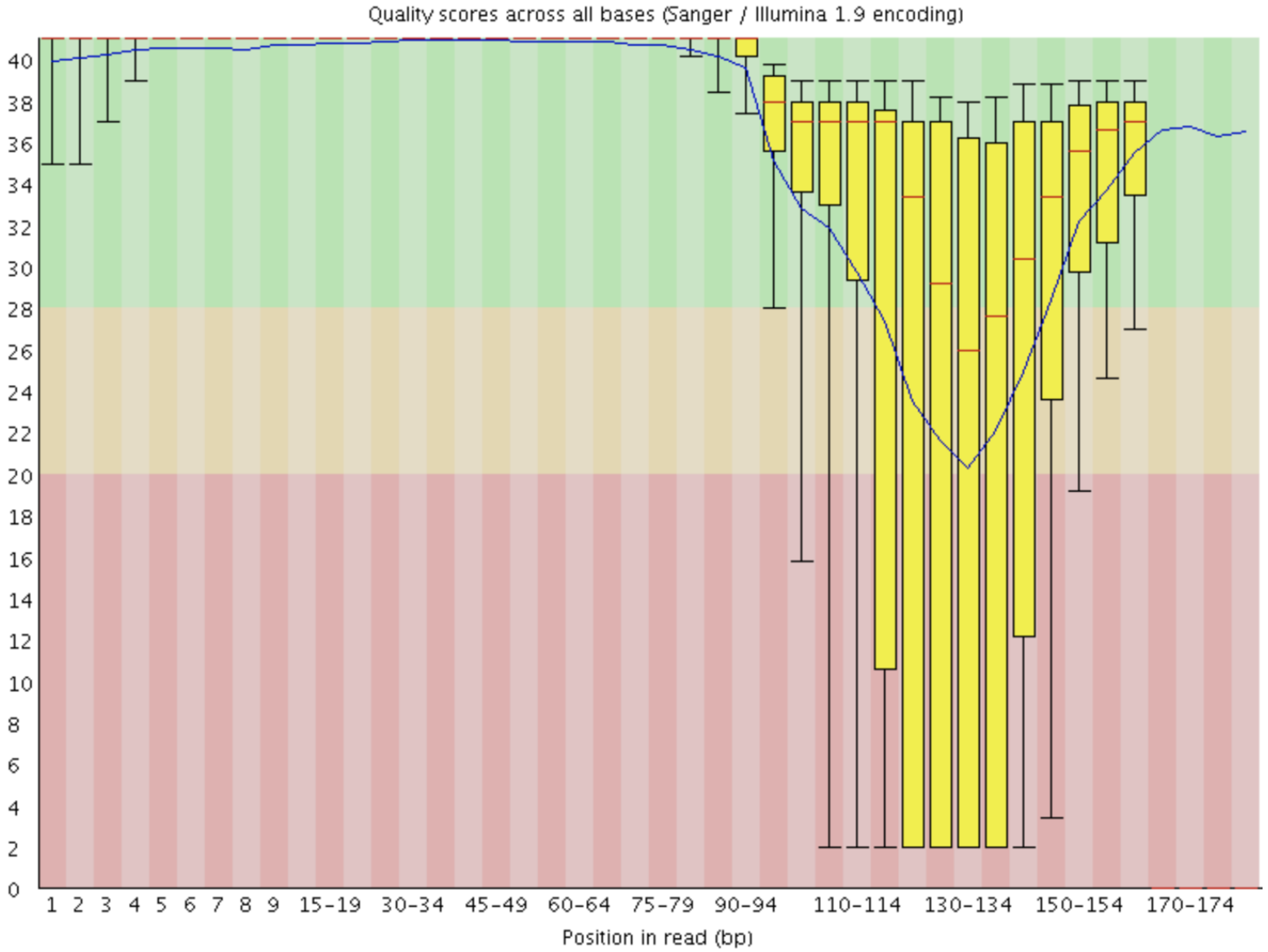
Depth of coverage in HTS



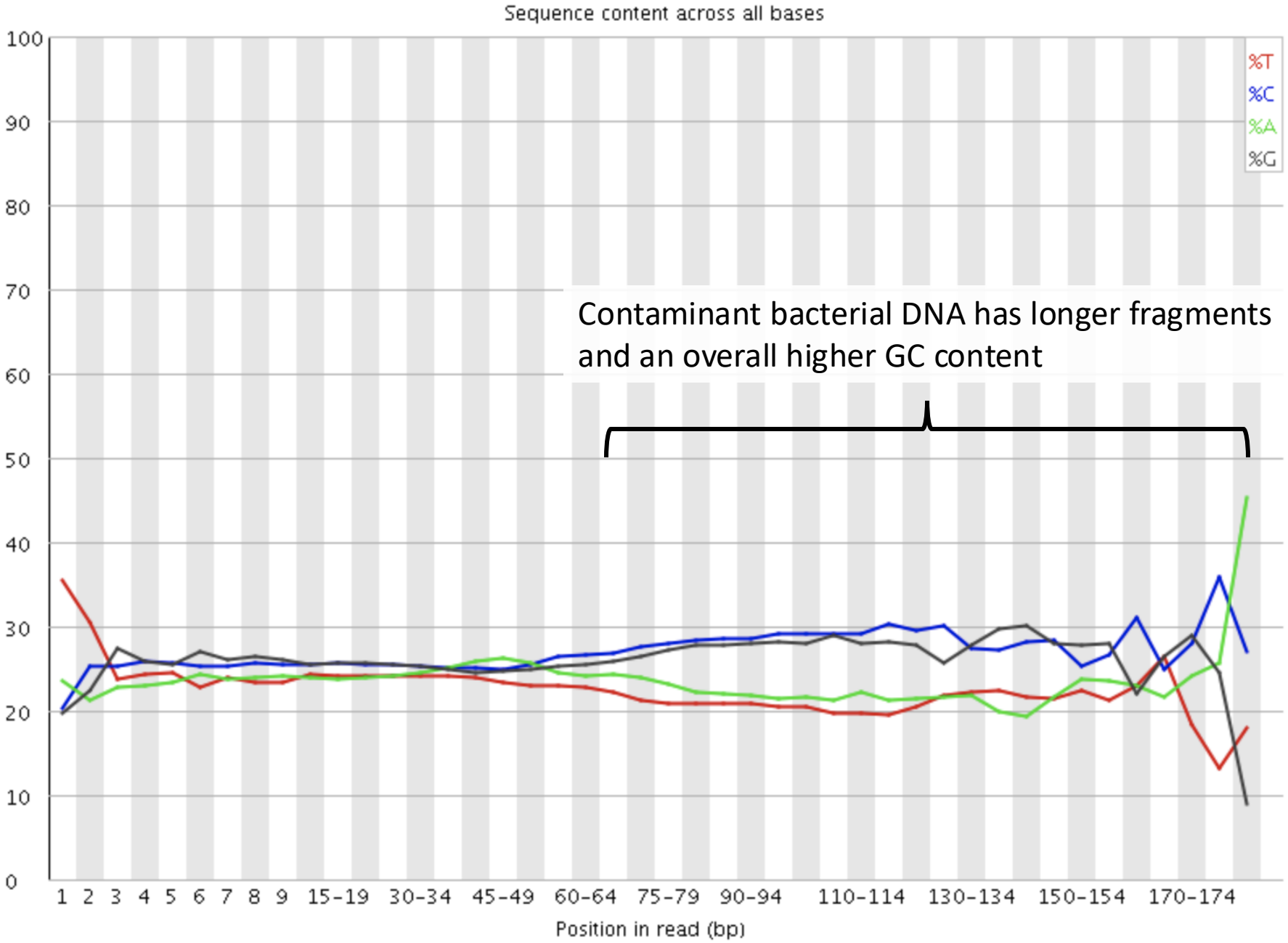
Deep sequencing of ancient DNA allows independent replication of variant sites to identify miscoding lesions and remove false positive results

Typical base quality distribution of ancient DNA in Illumina sequencing data

- Sequencing runs are typically longer than ancient DNA fragments
- Many sequencing molecules fail to extend properly in the absence of template
- Errors accumulate and light signal is harder for the sequencer's camera to interpret correctly
- Base quality scores decrease dramatically towards the ends of ancient sequencing reads



Typical higher GC content towards the end of sequencing reads

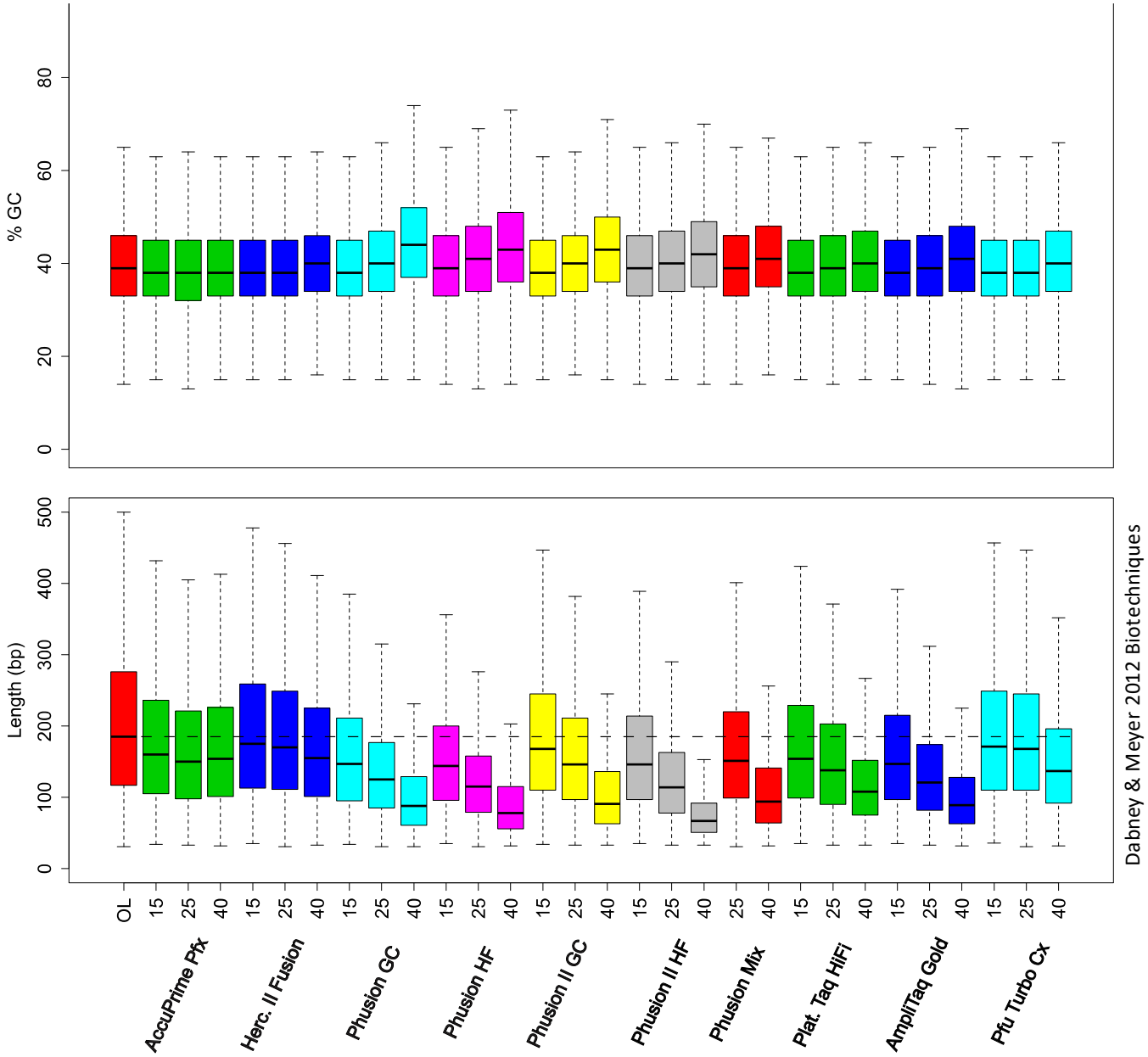


Data biases due to experimental procedures

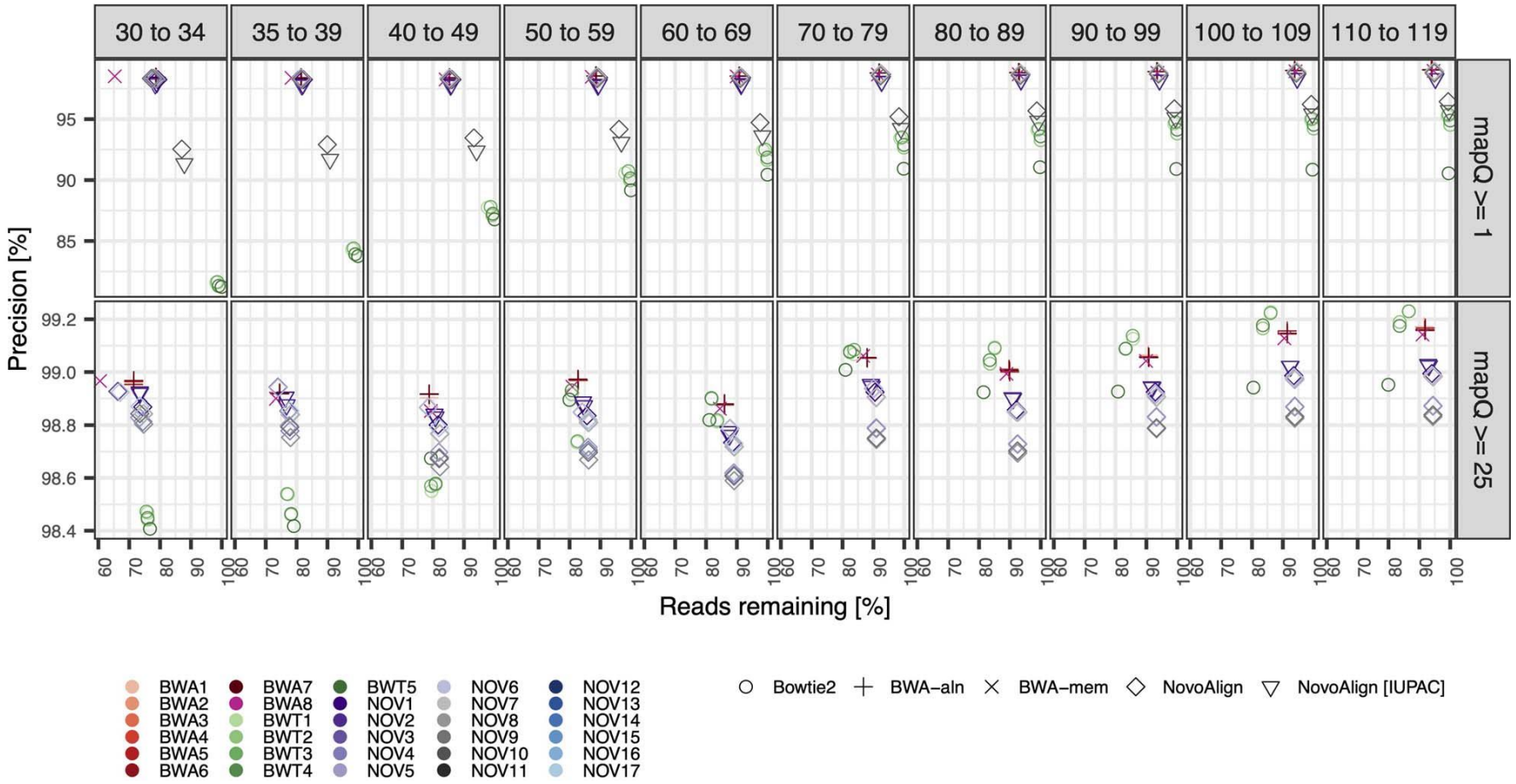
The performance of DNA polymerases used to perform PCR depends on GC content and fragment length

Some polymerases will amplify preferentially high GC-content DNA (i.e., microbial DNA)

Given the distribution of ancient DNA fragment lengths, length bias should be minimised



Impact of read mapping strategies when processing ancient DNA sequencing data



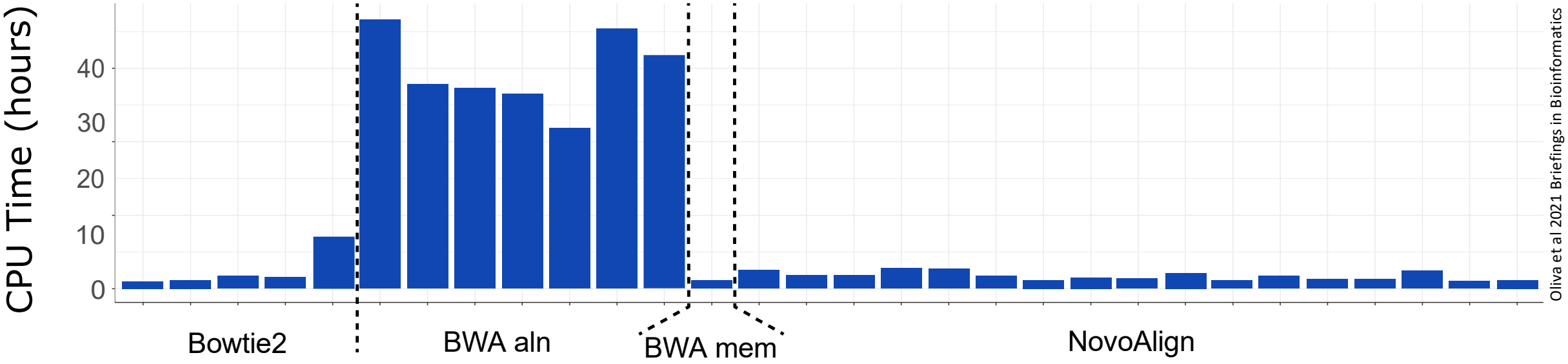
Oliva et al 2021 Briefings in Bioinformatics

Due to the short length of ancient DNA reads, mapping software performance varies for:

- Precision, where the longer the read, the more accurate the mapping
- The proportion of mapped reads

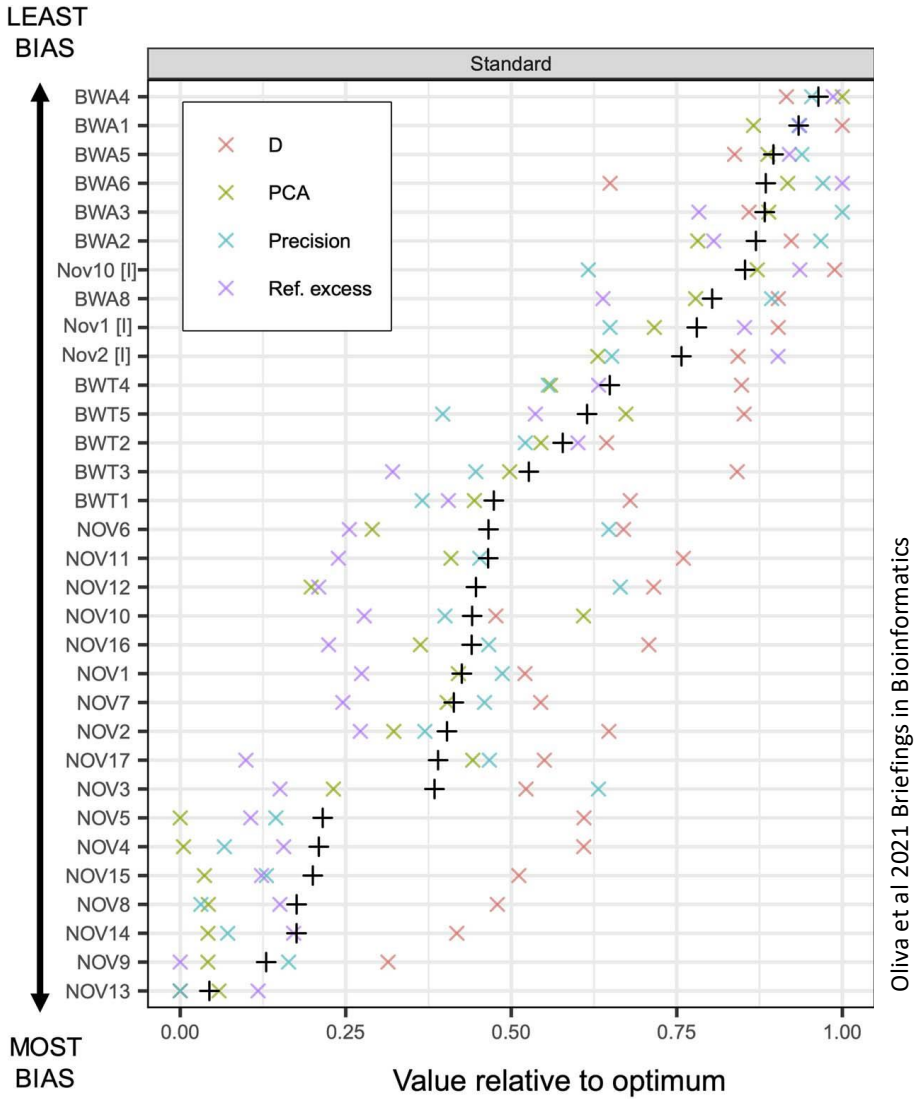
Filtering out reads with low mapping quality generally improves the mapping process

Impact of read mapping strategies when processing ancient DNA sequencing data



Reads mapping strategy must take into account CPU time, especially if resources are limited

Impact of read mapping strategies when processing ancient DNA sequencing data



- D: D-statistics estimate the level of relationship between test populations.
- PCA: PCA allows to visualise high complexity datasets in a 2D representation. The closer two individual datasets are in the PCA space, the more similar they are.
- Precision: mapping to the correct genomic location.
- Ref. excess: reference allele bias.

Reference bias leads to wrong population genetics inferences

Summary

- Post-mortem processes impact DNA integrity and survival
- We can observe characteristic patterns in ancient DNA sequencing data
- It is possible to repair ancient DNA damage experimentally
- Bioinformatic methods can help estimate the amount of contamination
- Mapping of ancient DNA sequencing data requires balancing between key performance indicators

Thank you for your attention!

Don’t forget to become a member of

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